Charles University

Faculty of Science

Study program: Botany

Branch of study: Geobotany

Bc. Thuy Lien Duongová

Effect of climate on germination and selection of different genotypes

Efekt klimatu na klíčení a selekci genotypů

Diploma thesis

Supervisor: Doc. RNDr. Zuzana Münzbergová, PhD.

Prague, 2018

Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, dne 13.8.2018

Thuy Lien Duongová

Acknowledgment

I would like to thank my supervisor Zuzana for her expert advice, kindness and never ending patience. I am grateful to meet such an enthusiastic and hard working person as her. She encouraged me during hardships and pushed me to my limits.

Many thanks to the team in the Institute of Botany of CAS, especially Andrea Jarošová and Mária Šurinová for showing me laboratory techniques. I would like to thank Ilona Jarošincová and Ilona Chmelařová for helping me with maintenance of the growth chambers during the semester.

Lastly, I would like to thank my family and friends for reminding me to smile during the whole process.

Abstract

Understanding the response of species to climate change and their ability to adapt is the key to describe the future development of plant communities. The aim of the study is to determinate intraspecific variability in germination of *Festuca rubra* from different original climates in response to novel climatic regimes. This study also observes if different climatic regimes lead to selection of different genotypes.

Festuca rubra is a widespread clonal grass occurring in the Northern hemisphere. The plant material comes from 11 localities distributed along a climatic grid of factorially crossed temperature and precipitation situated in western Norway. The project was carried out in growth chambers, where the germination of seeds was monitored in two different temperature conditions and in two moisture treatments. Germinated seeds were planted into pots remaining in the same treatment where they germinated. Seedlings from one Petri dish grew together in one pot. One population, from the coldest and the driest original locality, growing in the warm-wet and cold-wet treatments was genetically analysed using microsatellites.

Germination of the species was higher and faster in warm than in cold conditions, showing that germination of the species is enhanced by higher temperature. Germination was higher in the wet treatments than in the dry treatments. This means that seeds germinated as long as they had sufficient moisture. In the dry treatments, seeds went into dormancy and were able to germinate in optimal conditions later. Seeds originally from colder areas germinated in greater proportion in the warm-wet treatment. This suggests better germination of species originally from colder areas in global warming scenarios.

Genetic differences in initially identical genotype mixtures in two treatments were significant, suggesting possibility of selection of genotypes under different climate. Results showed unique information on how various environments can select genotypes from present variable genetic set in the population. Information available on this topic is however still very limited and more research is required.

Key words: genotype selection, climate change, germination, global warming

Abstrakt

K úspěšnému popsání budoucího vývoje rostlinných společenstvech je potřeba porozumět odpovědi druhů na klimatické změny a jejich schopnosti se těmto změnám přizpůsobit. Cílem práce je odhalit vnitrodruhovou variabilitu v klíčení v různých klimatických režimech druhu *Festuca rubra* (kostřava červená) původem z různých lokalit. Tato práce se dále zabývá efekty rozdílných klimatických režimů na selekci odlišných genotypů.

Festuca rubra je široce rozšířená klonální rostlina rostoucí na severní polokouli. Rostlinný materiál v této studii pochází z 11 lokalit na klimatickém gradientu v západním Norsku, kde jsou podmínky teploty a vlhkosti faktoriálně zkříženy. Studie probíhala v klimaboxech. Klíčení bylo rozděleno mezi faktoriálně zkombinované 2 režimy teploty a 2 režimy vlhka. Vyklíčená semena byla poté přesazena do květináčků ve stejných režimech jako ve kterých vyklíčila. Semenáče na jedné Petriho misce při klíčení poté dále rostly ve stejném květináči. Pouze jedna populace, původem z nejchladnější a nejsušší oblasti, rostoucí v teplém-vlhkém a studeném-vlhkém režimu klimaboxu byla geneticky zanalyzována za použití mikrosatelitů.

Semena klíčila rychleji a ve větší míře v teplém režimu než-li ve studeném režimu. To znamená, že klíčení druhu bylo podpořeno vyššími teplotami. Semena klíčila ve větší míře ve vlhkých režimech než v suchých. To znamená, že semena vyklíčila, pokud měla dostatek vlhkosti. V suchých režimech byla semena převážně dormantní a vyklíčila později po ukončení klíčící části práce v optimálních podmínkách. Semena původem z chladnějších oblastí gradientu vyklíčila ve větší míře v teplém-vlhkém režimu. To může znamenat, že druhy z chladnějších oblastí budou moci klíčit ve větší míře díky globálnímu oteplování.

Genetické rozdíly mezi identickými směsi semen ve 2 různých režimech byly signifikantní. Práce ukazuje unikátní výsledky o možné selekci genotypů, které jsou k dispozici v populaci, v různých klimatických podmínkách. Nicméně dostupné poznatky o selekci genotypů jsou velmi omezené a je potřeba tyto znalosti dále rozšířit.

Klíčová slova: selekce genotypu, klimatické změny, klíčení, globální oteplování

CONTENT

Abbreviations used:

PEG – Polyethylene glycol 6000

GI – germination index

T_{50} – time to 50% germination events

T – temperature (1 coldest, 3 warmest)

M – moisture (1 driest, 4 wettest)

AFLP – amplified fragment length polymorphism

PCR – polymerase chain reaction

SSR marker – microsatellite marker

CCA – canonical correspondence analysis

1 Introduction

Germination and seedling survival is one of the important processes to maintain and expand plant populations (Fenner & Thompson 2005). These characteristics are affected by abiotic factors such as temperature, moisture, light availability and soil composition. Also biotic factors such as pathogens and interactions between individuals play a significant role. For successful germination, requirements of a specific plant species need to correspond to actual environmental conditions. Germination is an irreversible morphological and physiological change of seeds. The first visible sign of germination is an elongating radicle and a sprout (Bewley 1997; Fenner & Thompson 2005). Seeds can germinate immediately after maturation or they can stay dormant and germinate after some time. Some of the seeds need time to mature and some need to break the dormancy for example by disruption of seed coats or by effects of low temperature (Baskin & Baskin 2014; Bewley 1997). Another type of dormancy inhibits germination when the seed is ready to germinate but the environment is not suitable. In temperate regions, germination usually takes place in spring thanks to higher temperatures and sufficient moisture (Baskin & Baskin 2014). Drought as well as extreme moisture can inhibit germination (Springfield 1968; Heydecker & Orphanos 1968).

Climate has been changing since the formation of the Earth's atmosphere. Nowadays, changes in the climate are mostly caused by human activity. Global warming is caused by increase of greenhouse gasses (carbon dioxide, methane, nitrous oxide etc.), which absorb heat from solar radiation and do not let the heat emit back to the universe (Houghton 1998). Changes in temperature and precipitation are the most noticeable. Temperatures of the atmosphere are increasing and will increase even more in the future, causing melting of glaciers and reduction of snow cover mostly on the Northern hemisphere (McCabe & Wolock 2010). Snow cover acts as a thermal insulation of the ground and without it the soil freezes deeper affecting biotic components of the soil (Körner 2003). It is predicted that by 2080 temperature in Europe will increase by 1.5-4.0°C compared to 1980 (IPCC 2014). With temperature increase, the evaporation of water also increases, leading to increased precipitations in some areas of the world (Wetherald 2002). Regions where climate change will strike most are arctic and mountain areas (Beniston *et al.* 1997; Beniston 2003; IPCC 2014). Alpine ecosystems are considered to be sensitive to global warming because they are characterised by low temperatures and low human influence (Körner 2003). Also, if the plants reach the top of the mountain they will not have anywhere higher to migrate. High mountains and arctic areas are under snow and ice cover. If snow and ice thaw, it will enhance global

warming because these areas act as cooling factors of the atmosphere. But it will prolong the Arctic growing season for plants (IPCC 2014; NSIDC 2018). Thanks to the complexity of topography and rapid changes in temperature and precipitation over short distances, mountains provide unique locations to study signals of climatic change and its impact on hydrological, ecological and societal systems (Beniston 2003).

Plants can react to climate change by migration, phenotypic plasticity and genetic adaptation. It is clear that some species will not be able to cope with these rapid changes and will face the danger of extinction (Jump & Peñuelas 2005). Species that reproduce slowly and disperse poorly, and those which are isolated or are highly specialized, will be highly sensitive to seemingly minor stresses (McNeely *et al.* 1990). Species can migrate to higher altitudes to escape the temperature increase. In the present, we can observe shift of boreal and alpine species to higher altitudes (Harsch *et al.* 2009; Lenoir *et al.* 2008). However, if the temperature increase will be too extensive, species will not have enough space to move further upwards or they will not be able to migrate fast enough. In the study by Parolo & Rossi (2008) a calculation of altitudinal migration rates along a continuous elevation gradient of the Bernina Peak in the Central Alps of more than 700 m distinguished rapid and slow migrants and species that did not migrate to higher elevations (excluding those that were already at the peak). This implies differential abilities of species to cope with an increasingly warmer climate and thus, a different threat of extinction (Parolo & Rossi 2008). Long-term upslope range-shifts driven by temperature increases have been recorded in many montane species but, in numerous cases, range-shifts are lagging behind climate change (Chen 2012). Response via phenotypic plasticity involves expression of different morphology or physiology by plants of the same genotype under different environment (Nicotra *et al.* 2010). Phenotypic plasticity can be seen as changes in seed traits (including germination), plant height, leaf mass per area, flowering time, etc. In the short term, the plastic response of existing genotypes will be of particular importance in determining plants' persistence under climate change (Nicotra *et al.* 2010; Ghalambor *et al.* 2007). It is needed to distinguish between phenotypic plasticity and genome-based changes under climate change although it is very complex. Plasticity and evolution occur simultaneously, and these are not alternative or mutually exclusive responses (Franks *et al.* 2014). To study trait plasticity, organisms need to be transferred to uniform conditions. One of the approach to test phenotypic plasticity is to compare plant individuals growing in uniform conditions to plant individuals growing on the site of origin of used plants. To test genetic differentiation, plants individuals from different populations and conditions growing under uniform conditions can be compared between each other.

Genetic adaptation is as a result of evolutionary change of organisms to fit into their environment. Evolution is by definition a change in allele frequencies and therefore sufficient heritable genetic variation must exist for evolution by natural selection to occur (Ghalambor *et al.* 2007). Adaptation should help organisms to have higher chance of surviving under new circumstances. Jump & Peñuelas (2005) suggested that adaptation might be more important than migration and phenotypic plasticity. Changes in climate can select certain genotypes from the ones available in the population (Kelly *et al.* 2003). Climate change creates selection pressure on traits important for survival of the species population. However, genetic adaptation may not produce changes fast enough to mitigate the effects of climate change. To see effects of climate on population we can observe population exposed to different climates either in natural or experimental conditions. Along a gradient, the divergence between populations may be influenced by differences in the selective pressures imposed by different ecological environments, neutral evolutionary processes or both (Still *et al.* 2005). However, we lack in-depth knowledge about the micro evolutionary adaptation of plants and evolutionary potential of plant populations (Gienapp *et al.* 2008).

Observing climatic changes and responses of plant species can help us predict the population dynamics in the future (Morin & Thuiller 2009). It can lead us to wisely invest in biodiversity and ecosystems protection (Walther *et al.* 2002). Protecting organisms from extinction can help us keep the benefit from ecosystem services. For example, plants provide oxygen, nutrient cycling and biomass not only as an economical resource. We also protect the biodiversity for aesthetics and moral reasons. The World Conservation Union (IUCN) recognizes the need to conserve the biological diversity on Earth (McNeely *et al.* 1990).

1.1 Germination and seedling survival

During the last 150 years, the global warming has been changing the germinability of seeds, the timing of germination, the rate of germination and the seedling recruitment patterns. Germination and seedling establishment are expected to be more sensitive to climate changes than adult stages (Fay & Schultz 2009). Temperature increase alone without increase of precipitation can reduce germination but with sufficient level of moisture and higher temperature in the spring plants can profit from the change and higher number of seeds can germinate (Walck *et al.* 2011; Liu *et al.* 2011). This suggests the possible interaction between temperature and moisture. The interaction was already previously shown in adult plants

(Meineri *et al.* 2013; Münzbergová *et al.* 2017) and now it is needed to be observed it in germination as well.

As said above, Arctic and mountain regions will be affected by global warming the most. Results from Müller *et al.* (2011) confirmed that seeds of arctic species have higher germinalibity and viability due to global warming. There is a threat that plants, which need to break dormancy through low winter temperatures, will not be able to germinate because of too warm winters (Fenner & Thompson 2005). On the other hand, seeds of several species from the subarctic area germinated earlier in the spring time as a reaction to the absence of the snow and prolonged their vegetative period (Milbau *et al.* 2009). Graminoids might profit the most from the temperature increase. For example, *Deschampsia, Carex, Luzula* and *Poa* germinated better under warmer conditions (Shevtsova *et al.* 2009; Mondoni *et al.* 2012; Fernandez-Pascual *et al.* 2015).

Most of the studies observing germination under different temperature look at interspecific differentiation. Species germination requirements differ between species and are linked to their demand on surrounding conditions. Most species, however, germinate better in warmer conditions in case of sufficient moisture. Studies observing intraspecific differences in seed germination are in minority (Cavieres & Arroyo 2000; Graae *et al.* 2008; Fernandez-Pascual *et al.* 2015). Intraspecific differences in germination are also influenced by seed origin and origin of their maternal plant, genetic variation of the population and phenotypic response to the environment (Sultan & Spencer 2002; Qaderi & Cavers 2002). Cavieres & Arroyo (2000) mentioned that there is variability between seeds of the same species in germination response to different snow cover. Intraspecific comparisons showed that seeds from warmer localities germinated better under warmer regimes in comparison with regimes with lower mean temperature during the day (Fernandez-Pascual *et al.* 2015; Graae *et al.* 2008). Also individuals of the same species but from lower altitudes were more drought tolerant and germinated better than those from higher altitudes above the tree line in the Alps (Walder & Erschbamer 2015). But De Vitis *et al.* (2014) suggests that the thermal germination behaviour may be affected by the maternal environment of seed production within one generation. They recommend using seeds produced in the same environment in which they will be germinating to reach the best germinalibity.

Conditions suitable for germination are not always suitable for seedling survival or adult plants (Lloret *et al.* 2004). Generally, limited moisture reduces the chance of survival of young seedlings. Changes of the climate will mostly affect the timing of germination and

seedling survival. Both attributes might shift from spring activity to autumn activity or start very early in the spring (Mondoni *et al.* 2012).

1.2 Selection of genotypes under climate change

As mentioned above, rapid adaptation is an important process allowing species to respond to changing climate. Whether a plant in terrestrial ecosystems is able to adapt to the environmental changes depends on its population genetic characteristics and phenotypic plasticity (Gao *et al.* 2018). However, the ability of plants to adapt to changing climate has not yet been sufficiently examined (Parmesan 2016). We can approach this problem by transplant experiments in gardens and germination in growth chambers exposing plants to novel climatic condition (Koti *et al.* 2005; Gordon & Rice 1998; Wang *et al.* 2017) or with the help of laboratory methods to see the actual genotypes and changes in genetic composition of the populations after being exposed to novel climatic conditions (Ravenscroft *et al.* 2015).

Selection in any population will favour individuals that produce a phenotype appropriate for the local environment. Genetically distinct locally specialized ecotypes are expected to be selected when a single genotype is represented with a single phenotype and different phenotypes are required in different environmental states (Sultan & Spencer 2002). In a widespread species, the conditions for divergence and local speciation are likely to exist in populations at the geographical and ecological edges of the species distribution (Levin 2003). For example, many tree species have shown strong evidence of local adaptation to climate in provenance experiments, which are garden experiments testing the effects of the place of seed origin on tree survival, growth, and other phenotypic traits (Franks *et al.* 2014). It was demonstrated that different environments support establishment of different genotypes of trees (Jump *et al.* 2006). Phenotypic plasticity can be distinguished from genetic differentiation, which includes local adaptation and non-adaptive genetic changes, depending on whether quantitative trait differences among populations *in situ* disappear by raising individual plants from these populations under the same conditions - e.g. using common garden experiments (Bresson *et al.* 2011; Martin *et al.* 2007). Moreover, if quantitative trait differences *in situ* are larger than those in common garden experiments, they might be controlled by the combination of phenotypic plasticity and genetic differentiation (Riordan *et al.* 2016; Gao *et al.* 2018). Comparing meaningful correlations between the values of quantitative traits in the common garden and the environmental variables in their original

habitats could predict local adaptation to the selection of environmental changes (Bresson *et al.* 2011).

Common garden experiment of Qaderi & Cavers (2002) observed germination and obtained much higher final germination percentages from seeds produced in the warm summer of 1999 and much lower ones in the cool summer of 1997. Their observation also revealed that there were consistent differences among *Onopordum acanthium* populations regardless of the collection year and date, and this supports the possibility of genetic differences among the populations. Seeds of two different populations but produced under the same conditions over 4 generations still had large differences in germination response, suggesting genetic differences again (Qaderi & Cavers 2002).

Several recent studies studying effects of changes of conditions using growth chambers and greenhouses demonstrated the importance of rapid evolution in annual plants. Koti *et al.* (2005) compared 6 different genotypes of soybean. They planted genetically different seeds into new conditions in growth chambers. They mainly focused on morphological changes of reproductive organs, but they also mentioned that some of the planted genotypes were more sensitive to the change and some were in contrast more tolerant towards stress events (e.g. effects of temperature, ultraviolet-B radiation). The difference in sensitivity of different genotypes to climate suggests potential for their selection. Another study by Nevo *et al.* (2012) compared 10 populations of *Triticum dicoccoides* and 10 populations of *Hordeum spontaneum* across Israel, sampling them in 1980 and again in 2008. They compared the plants from germination up until flowering in the greenhouse with different water regimes. Results showed faster flowering time and allele reduction in populations from 2008 suggesting evolutionary changes in the populations. Wang *et al.* (2017) compared 26 cultivars of fine fescue species (*Festuca*) in prolonged heat or drought stress in growth chambers. Results from this study demonstrate that fine fescues were more sensitive to heat stress than drought stress, and that there were greater genotypic variations in heat tolerance than drought tolerance within the fine fescue species. There was greater potential for improving heat-tolerance due to greater sensitivity to heat stress and greater genotypic variation of heat tolerance.

An amplified fragment length polymorphism (AFLP) laboratory study of Ravenscroft *et al.* (2015) showed similar patterns for perennial herbs. They studied the effect of experimental drought and warming in grasslands over 15 years and demonstrated a significant genetic differentiation of plant populations exposed to the different treatments over this period. Analyses revealed a consistent signature of selection associated with experimental climate

treatments at individual AFLP loci in *Plantago lanceolata*. It shows genetic response of mature populations of the perennial plants to climate change. In the study by Still *et al.* (2005) they also used AFLP laboratory method on *Echinacea angustifolia* along a climatic gradient. Results showed about 60% of the genetic variation was found within populations and 20% among populations (Still *et al.* 2005). The data supports an isolation-by-distance restriction in gene flow that is independent of annual mean precipitation. However further studies on species potential to adapt to novel conditions are needed to understand to what extend such an ability is general.

1.3 Choosing the model plant

Festuca rubra was chosen as a model plant. It is a widespread clonal grass species in Northern Hemisphere. In Central and Northern Europe, it is a dominant species in higher altitudes. It is a perennial Eurasian grass, which can spread through seeds or vegetative growth. Seed germination can be enhanced by cold stratification but it is not necessary (Baskin & Baskin 2014). This plant is very variable and can adapt to a wide range of climatic conditions. Plants falling into morphologically distinguishable categories are often inconsistently classified as both species and subspecies showing local adaptations (Dirihan *et al.* 2016; Sampoux & Huyghe 2009). *Festuca rubra* is mostly a hexaploid and populations of *Festuca rubra* are very genetically diverse (Sampoux & Huyghe 2009). Most of the genetic variation occurs within populations (Šurinová *et al.* submitted). The performance of the plant was strongly determined by climate of origin, but the populations also show high plasticity and the type and intensity of the plastic response depended on the climate of origin (Münzbergová *et al.* 2017). Previous work did not study germination characteristics of *Festuca rubra*, which might be crucial for genotype selection. Also, this species is a dominant in higher altitudes, which will be affected by global warming the most.

1.4 Aims

In this study I would like to reveal potential for selection of genotypes under different climatic conditions in species *Festuca rubra*. This study is unique because it will test different combination of temperature and moisture on a widespread clonal grass with great ability of phenotypic plasticity. It will show whether the selection of genotypes is an important part in the species survival in the future climatic conditions. I ask the following questions:

1) What is the effect of temperature and moisture on species germination and do these two factors interact?

2) Does the effect of temperature and moisture depend on origin of the population? How strong is this influence?

3) Can we identify signatures of selection in stands established from genetically identical seed mixtures under different climates?

2 Methods

The study uses unique SeedClim design from University of Bergen, Norway (Meineri *et al.* 2013; Münzbergová *et al.* 2017). This design consists of 12 localities situated in western Norway on the climatic gradient. On these 12 localities mean annual temperatures (7.5, 9.5 and 11.5°C) and mean precipitation (600, 1300, 2000, 2700 mm) are factorially combined. Other factors like latitude, length of the daylight and abiotic conditions are similar on these localities.

On 11 localities separate ramets of *Festuca rubra* representing different genets were collected. On the 12th locality *Festuca rubra* does not occur. This locality represents colder area with intermediate moisture (T1M2 - Lavisdalen). I used plants that were brought to the Czech Republic from Norway and were grown in the experimental garden. Each ramet was grown in a separate pot, where it could spread through clones without mixing with other genetically different individuals. At the beginning of the summer, before flowering of the plants, pots from the same population were put under the same cage with fine mesh. So the individuals in the population would only cross with another individual in the same population but not between populations. During August (2016) we collected seeds. Seeds from different mother plants were kept separately in paper bags.

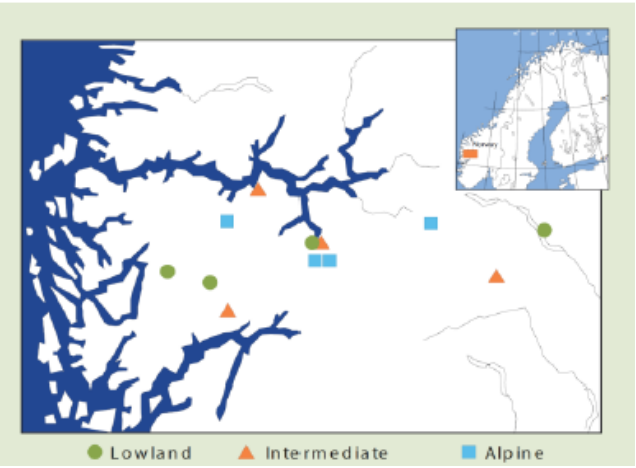

Figure 1 – Study localities in western Norway

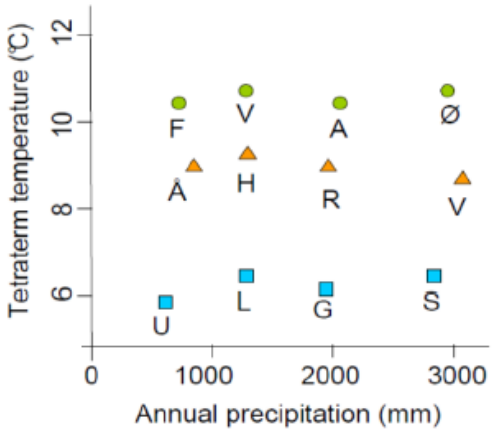

Figure 2 – Gradients of annual temperature and precipitation on the study localities

2.1 Germination experiment

Germination of seeds took place in two growth chambers. They represented 2 temperature regimes, warm and cold. The cold regime was set to 3°C/12.5°C with mean temperature 8°C during the day. The warm regime was set to 3°C/24.5°C with mean temperature 13.5°C during the day. The daylight period lasted from 6-22 o'clock. In every growth chamber, there were 2 different moisture regimes, wet and dry. Temperature and precipitation settings were established to mimic real field data provided by our Norwegian colleagues set to the extreme conditions from the climatic gradient.

From each population, I had in total 25 different mother genotypes per population, from which 14 to 20 maternal plants were chosen, which produced at least 20 seeds. The proportion of seeds used in the experiment was estimated to correspond to the actual number of seeds from maternal plants. Plants with more seeds have a higher number of seeds used in the experiment. At this point, only maternal genotype of seeds is known, the paternal genotype is not known, but it comes from the same population. A mix of 20 seeds from one population from different mother plants were put onto one Petri dish. In each case, 20 Petri dishes were composed of seeds from exactly the same maternal plants in the same proportions. These identical 20 dishes were divided between 4 treatments – 5 for each treatment (warm/cold x wet/dry). Another mixture of genotypes was created for another 20 Petri dishes of seeds from one population. This means I had two sets of dishes (40 dishes in total) for temperature and precipitation regimes from each population. I will refer to these two mixtures of genotypes as Set A and Set B (Fig. 3).

In several populations not enough of seeds were produced. I was forced to plant seeds only in 2 regimes and not 4 – only warm-wet and cold-wet combinations. That means 2 sets of dishes were used - only 20 Petri dishes in total. Populations with only 2 regimes are T1M3, T1M4, T2M1 and T2M4. Result from these populations will be absent in several tables. They will be marked as N/A.

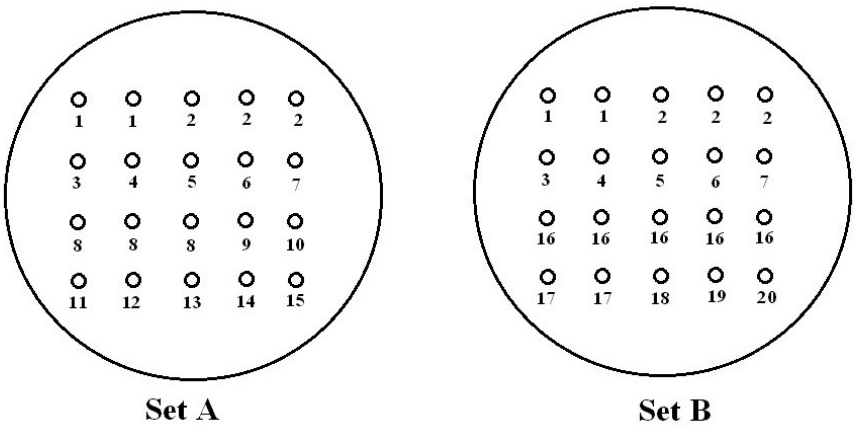

Figure 3 – Example of 20 seeds on a Petri dish from one population, numbers match maternal genotypes, 2 sets (A,B), each set is repeated 20 times.

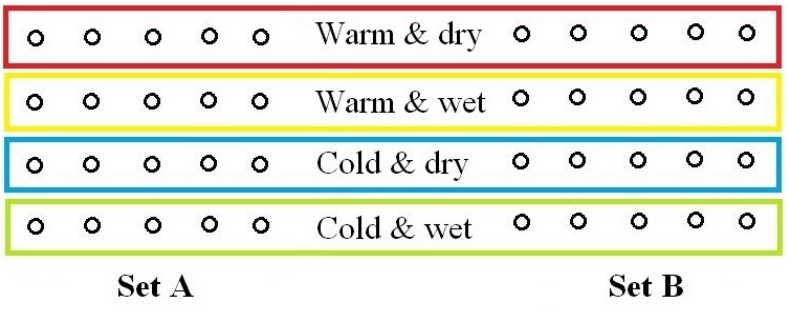

Figure 4 – 2 sets of 20 Petri dishes from 1 population dividend into 4 regimes

Dishes were watered with distilled water in wet regimes. For dry regimes, solution of Polyethylene glycol 6000 (PEG) with water was used to simulate drought (Walder & Erschbamer 2015; Pérez-Fernández *et al.* 2006). The optimal PEG ratio was counted for used temperature modes from (Michel 1983). The drought matched -0.7MPa. Studies examining drought stress usually use range for example between -0.4 and -2MPa (Sadeghi *et al.* 2011). Dishes were left to germinate in the growth chambers. Dishes were checked once a week. Seeds with at least 2 mm visible long root or 2 mm long green sprout were considered as successfully germinated e.g. (Bernareggi *et al.* 2015; Mondoni *et al.* 2015). Seedlings were planted into pots (see more below). Mouldy seeds with decomposed embryo were removed from the dishes.

After the termination of the germination part, which was 8 weeks with no new seeds germinated in the growth chamber. The whole germinating part took 32 weeks. Seeds that did not germinate (mostly seeds from dry-cold treatment) were moved to warm growth chamber (3°C/24.5°C), PEG solution was washed out with distilled water and seeds were watered with

distilled water to check their viability via germination in more suitable conditions. Viability of the rest dormant seeds that did not germinate was tested with tetrazolium chloride (Cottrell 1947). The rate of germination, the speed of germination and seed dormancy can be deduced from records of the germination part.

2.2 Genotype selection experiment

For the second part, small pots were prepared (size 5×5×8cm) corresponding with the number of Petri dishes. Pots were filled with substrate of soil and sand in ratio 2:1. After successful germination seeds were planted into labelled pots and well watered with tap water. To be clear, seeds were planted in the pot right away in the same week they germinated. Seeds were gradually planted from the centre of the pot to the edges. Pots were watered from above when the seedlings were still about 3-5 cm small. After this phase, pots were watered from below. Wet and warm regime was watered to the extent, so the pots would always stand in 1 cm of water at least – approximately 8 litres per week for 95 pots. Wet and cold regime was watered with 6-7 litres per week. Dry regimes were watered also with tap water but less – approximately 2 litres per week for 95 pots.

Positions of pots were shifted randomly after every 4 weeks in the growth chamber. A pot with soil and a temperature and precipitation sensor (TOMST) was added to keep the exact record in every regime.

After 18 weeks from the start of the germination experiment the pots were fertilized with solution of fertilizer (Nitrogen (N) 8%, Nitrate (NO_3-N) 4%, Ammonium (NH_4-N) 4%, Phosphorus pentoxide (P_2O_5) 4%, Potassium oxide (K_2O) 6%, Iron (Fe) 0.02%, Boron (B) 0.01%, Copper (Cu) 0.01%, Manganese (Mn) 0.01%, Molybdenum (Mo) O.002%, Zinc (Zn) 0.002% in 500ml) and water in ratio 12ml/l for 95 pots. The height of the tallest stem was measured and then plants were cut down to height of 2 cm. Number of ramets was counted in every pot. Cut biomass of every pot was kept and weighed when dried. Plants in the growth chamber were fertilized again and then they were left to grow for another 14 weeks until cutting for genetic analyses.

For the genetic analyses, only the population from the driest and coldest area T1M1 (Ulvehaugen population) was used. Genetic material between 2 growth chambers was compared – warm-wet and cold-wet, which have different climatic conditions than the original locality. While it would be better to use cold-dry chamber instead of cold-wet, population T1M1 did not germinate during the germination part at all in the cold-dry chamber

representing the conditions of plant origin. And in the warm-dry few seeds germinated, but did not establish seedlings. Thus there were no samples to be analyzed. Every fourth ramet in one pot was cut after 28 weeks for genetic analyses. The reason behind not cutting every ramet in the pot was because *Festuca rubra* often spreads clonally. So, to limit analysing the same clone over again, every fourth ramet was cut. It was needed to cut healthy green ramet to successfully perform genetic analyses but at that time a big part of individuals were yellowing. Also, genetic analyses are financially demanding, so the number of analyses were reduced this way. Ramets were cut gradually from left side of the pot to the right side. Biomass was dried in silicagel after cutting. Sum of 356 samples were analyzed. Approximately 18 samples were taken from 1 pot.

Protocol which was successfully applied in Šurinová *et al.* (submitted) on mother plants of planted seeds was used for this study. For DNA isolation from dried leaves in silicagel DNeasy 96 Plan Kit (QIAGEN, Germany) was used. Quality and quantity of extracted DNA was measured by NanoDrop 2000 (Thermo Scientific, USA). Samples were analyzed with the use of 5 microsatellites loci (HVM20, HVM3, HVM4, B4-D6, B3-B8) characterized in publications Fu *et al.* 2006 and Lauvergeat *et al.* 2005. All of the mentioned primers were mixed into one multiplex PCR mixture. Multiplex PCR reaction contained 2.5 µl QIAGEN Multiplex PCR Master Mix, 0.25 µl of HVM 20 primers, 0.1 µl of HVM4 primers, 0.05 µl of HVM3, B3-B8, B4-D9 primers (10 µM each in initial volume) 0.5 µl H_2O and 20 ng of DNA dissolved in 1 µl TE buffer. An initial denaturation step at 95 °C for 15 min was followed by 40 cycles of denaturation (95 °C for 30 s), annealing (60 °C for 90 s) and extension (72 °C for 60 s) steps, and a final extension step at 72 °C for 10 min. A 1 µl aliquot of the PCR product was mixed with 11 µl of a 120: 1 solution of formamide and size standard GeneScan 500 LIZ (Life Technologies, USA). Fragmentation analysis was performed using the ABI 3130 Genetic Analyzer (Thermo Fisher Scientific, USA). Fragments length were determined by capillary gel electrophoresis using GeneMapper 4.0 (Life technologies, USA) and the peaks were scored manually afterwards.

Number of 5 microsatellites was proven enough to distinguish individual genotypes in Šurinová *et al.* (submitted) thanks to high polyploidy of the *Festuca rubra.* Šurinová *et al.* (submitted) identified 73 different alleles using these microsatellite markers in the maternal plants of seeds used in this study. Similarly, Pfeiffer *et al.* (2011) suggested that due to the larger number of alleles per locus, the discriminative power of a locus is potentially higher for polyploids than for diploids and the successful fingerprinting of polyploids requires

substantially fewer loci. Six loci were also used in other studies on polyploids e.g. Besnard *et al.* (2008) and Teixeira *et al.* (2014).

2.3 Analyses

All data were analyzed in R programme (version 3.3.1).

2.3.1 Analyses of germination part

Seeds that did not germinate are considered as dormant seeds in calculations below. Their viability was checked by germination in optimal conditions or colouring them with tetrazolium chloride. I analyzed the proportion of viable seeds (germinated plus dormant seeds) in all seeds. And the proportion of dormant seeds in viable seeds was also counted.

To evaluate the speed of germination, germination index (GI) and time to 50% germination events (T_{50}) were calculated.

GI was calculated with formula from study Liu *et al.* (2014):

$$GI = \sum \left(\frac{n_i}{t_i} \right)$$

where n_i is the cumulative number of germinated seeds in time t_i (in our case one-week intervals)

T_{50} was calculated by following formula in Sadeghi *et al.* (2011):

$$\mathbf{T50} = \mathbf{ti} + \frac{\{(N/2) - ni\}\ (ti - tj)}{ni - nj}$$

N is the final number of germinated seeds and n_i and n_j are cumulative number of seeds germinated by adjacent counts at times (weeks) t_i and t_j when $n_i < N/2 < n_j$ (Sadeghi *et al.* 2011).

GI measures the initial slope of the germination curve - greater GI indicates faster initial germination. T_{50} covers the complete germination curve. Lower T_{50} means overall faster germination.

Measurements of germination characteristics were compared between same populations in different growth chambers and also compared between different populations in the same regime. All statistical analyses were done in the R programme (version 3.3.1). For proportion of germination, T_{50}, proportion of dormancy and viability generalized linear model and then ANOVA Chi-square test were used to analyze the differences. Gamma distribution

of proportion of germination, T_{50}, proportion of dormancy and viability was considered in the model. For GI linear model and ANOVA F-tests were used. Binomial distribution of GI was considered in the model. For ramets, height of plants and weight of biomass linear model was used. Due to distribution of number of ramets and weight of biomass, these data were modified. Values were squared. To test differences between original populations paired sample tests were used to test the interaction of moisture and temperature among temperature of growth chambers.

2.3.2 Analyses genetic part

Only population T1M1 from warm-wet and cold-wet treatment was analysed. This population has originally extreme conditions on the gradient, representing coldest and driest climate, which is in contrast of conditions where seeds germinated in the study and were used for genetic analyses. In the cold-dry chamber seeds did not germinate at all and in the warm-dry seeds did not establish any seedlings. Thus there were no samples to be analyzed. 10 pots from each treatment with the same genotype mixture were compared. Presence of all alleles in the genotype of every sample was recorded. Then I summarized the data for all ramets within a single pot and thus obtained information on frequency of alleles in each pot. Analyzes were calculated from all the single ramets between the growth chambers and from the whole pots among the growth chambers. In both variations analyzes were done within each genotype mixture (set A and B).

Statistical genetic analyses were performed in R program using vegan package. Genetic differences between the same genotype mixtures but in different climate were analyzed using multivariate canonical correspondence analysis (CCA). I used the CCA to test the effect of treatment (cold-wet vs. warm-wet), mixture of genotypes (Set A or Set B) and their interaction on genetic composition within the pots. To test the effect of the mixture, treatment was used as a covariate to filter out its main effect. The same was done for effect of the treatment, but mixture of genotype was as a covariate. For the interaction, main effects of the mixture and treatment were used as a covariate. Significance of the effects was tested with Monte Carlo permutation test with 999 permutations.

The genetic data were also analysed using an alternative approach. To do this, I calculated genetic differentiation between the treatments using the F_{ST} value in the Polysat package in R programme. I tested significance of the F_{ST} using 10 000 permutations of the data. First, I permuted all the single ramets between the growth chambers, in the second

version I only permuted the whole pots among the growth chambers. In both cases, the permutations were done within each genotype mixture (set A and B) separately.

3 Results

3.1 Germination characteristics

3.1.1 Germination rate

Populations from coldest and wettest localities germinated best from all of populations, followed by mid temperature populations (see Fig. 5). Warm-wet treatment had the highest rate of germination from all populations (87%-99%, see Fig.6), followed by cold-wet treatment (80%-93%, see Fig.7). Germination in these two treatments was comparable in all populations (Tab.1). Warm-dry (10%-53%, see Fig.8) treatment and cold-dry (2%-13%, see Fig.9) treatment had low rates. In warm-dry and cold-dry treatment, populations from the warmest localities germinated best. T3M4 did not germinate at all in the warm-dry treatment (Fig. 8). T1M1 and T3M4 did not germinate in the cold-dry treatment (Fig. 9).

Temperature of the original population had significant effect on germination rate. Seeds of cold origin germinated better in the warm treatment. Also, temperature and moisture of the growth chamber had significant effect on germination rate. Seeds germinated better in warm-wet and cold-wet treatment. Interaction between temperature and moisture of the growth chamber had significant effect (Tab. 2).

Population	overall	warm-wet	cold-wet	warm-dry	cold-dry
T1M1	0.47	0.99	0.93	0.03	0
T1M3	0.81	0.91	0.81	N/A	N/A
T1M4	0.92	0.93	0.92	N/A	N/A
T2M1	0.91	0.95	0.91	N/A	N/A
T2M2	0.43	0.87	0.82	0.23	0.06
T2M3	0.88	0.97	0.88	N/A	N/A
T2M4	0.50	0.98	0.93	0.49	0.11
T3M1	0.45	0.97	0.88	0.16	0.05
T3M2	0.36	0.94	0.93	0.36	0.13
T3M3	0.57	0.97	0.94	0.54	0.20
T3M4	0.46	0.98	0.93	0	0

Table 1 - Rate of germination across treatments - mean values of germination rate. Populations T1M3, T1M4, T2M1 and T2M3 did not have seeds in warm-dry and cold-dry treatments – marked as N/A

Df error = 276	RATE OF GERMINATION		GERMINATION INDEX		T50		VIABILITY		DORMANCY	
	Deviance	p	F value	p	Deviance	p	Deviance	p	Deviance	p
M.pop	0.000	0.966	1.594	0.208	39.2	**0.046**	0.002	0.519	39.2	**0.046**
T.pop	190.550	**<0.001**	73.030	**<0.001**	801.9	**<0.001**	0.019	**0.034**	801.9	**<0.001**
M.clim	2904.720	**<0.001**	2157.298	**<0.001**	8365.8	**<0.001**	0.149	**<0.001**	8365.8	**<0.001**
T.clim	191.360	**<0.001**	248.385	**<0.001**	16.1	0.201	0.018	**0.038**	16.1	0.201
M.pop:T.pop	0.160	0.688	21.614	**<0.001**	63.2	**0.011**	0.049	**0.001**	63.2	**0.011**
M.pop:M.clim	1.240	0.265	0.245	0.621	475.7	**<0.001**	0.002	0.441	475.7	**<0.001**
T.pop:M.clim	1.030	0.311	0.092	0.762	38.4	**0.048**	0.010	0.125	38.4	**0.048**
M.pop:T.clim	0.060	0.805	4.696	**0.031**	18.3	0.172	0.000	0.848	18.3	0.172
T.pop:T.clim	3.820	0.051	15.239	**<0.001**	26.8	0.099	0.020	**0.031**	26.8	0.099
M.clim:T.clim	4.310	**0.038**	47.525	**<0.001**	17.3	0.184	0.127	**<0.001**	17.3	0.184
M.pop:T.pop:M.clim	7.030	**0.008**	4.011	**0.046**	103.6	**0.001**	0.001	0.707	103.6	**0.001**
M.pop:T.pop:T.clim	7.010	**0.008**	0.235	0.629	2.3	0.628	0.009	0.133	2.3	0.628
M.pop:M.clim:T.clim	0.000	1.000	0.707	0.401	49	**0.025**	0.001	0.626	49.0	**0.025**
T.pop:M.clim:T.clim	0.000	1.000	0.216	0.643	35.4	0.057	0.004	0.306	35.4	0.057
M.pop:T.pop:M.clim:T.clim	0.000	1.000	0.078	0.780	0.1	0.920	0.001	0.589	0.1	0.920

Table 2 - ANOVA test results - Significant results are bold. Df – degrees of freedom. M – moisture, T – temperature, pop – original population, clim – climate of growth chamber

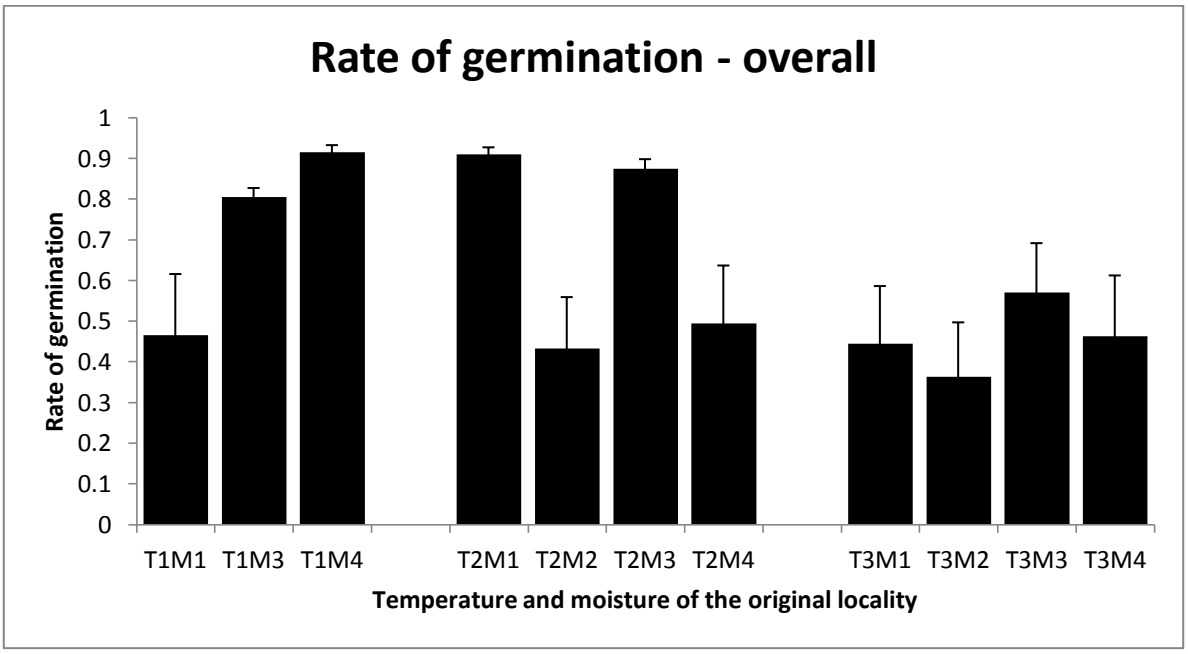

Figure 5 - Rate of germination – overall preview. Columns represent 11 original populations. Height of the column is the mean proportion + SE of germination of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1 is the driest, M4 the wettest.

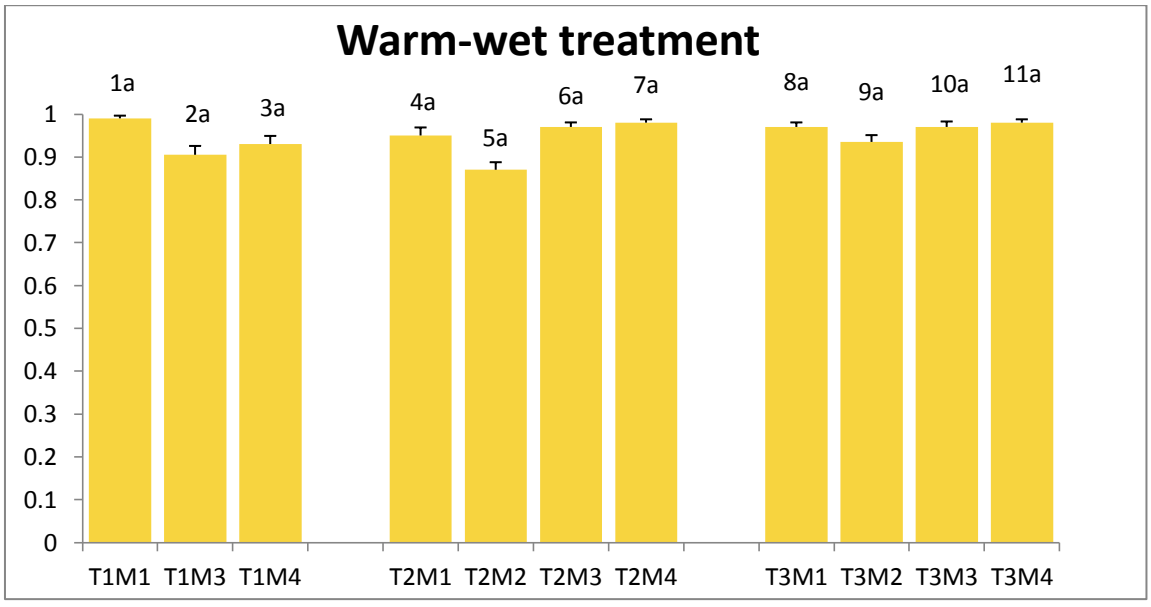

Figure 6 - Rate of germination in the warm-wet. Columns represent 11 original populations. Height of the column is the mean proportion + SE of germination of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1 is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population.

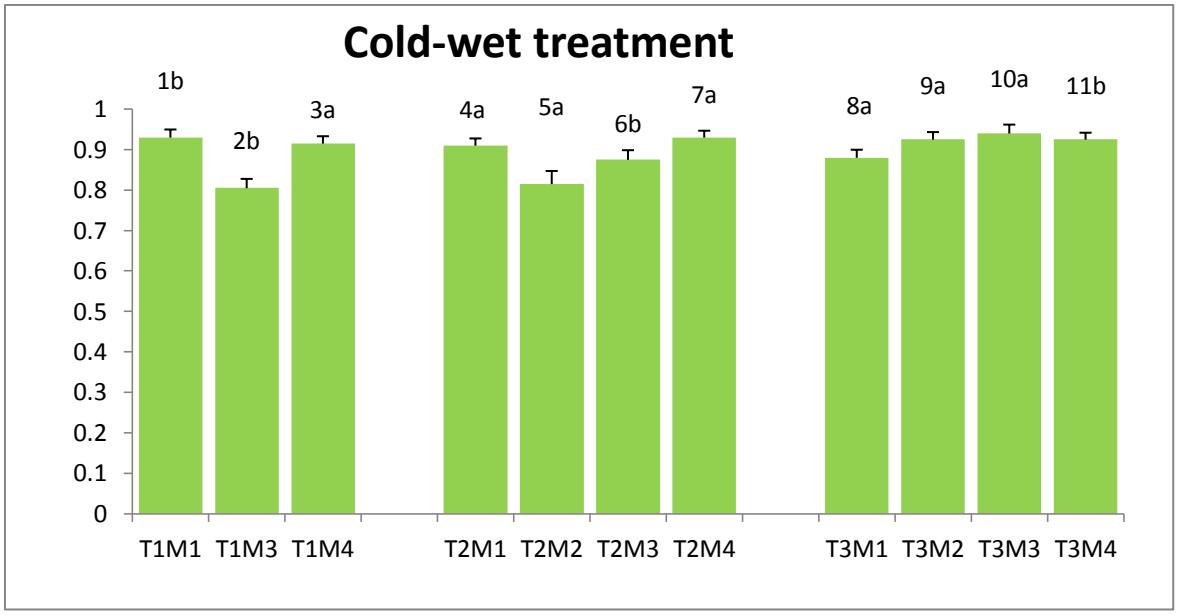

Figure 7 - Rate of germination in the cold-wet. Columns represent 11 original populations. Height of the column is the mean proportion + SE of germination of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population.

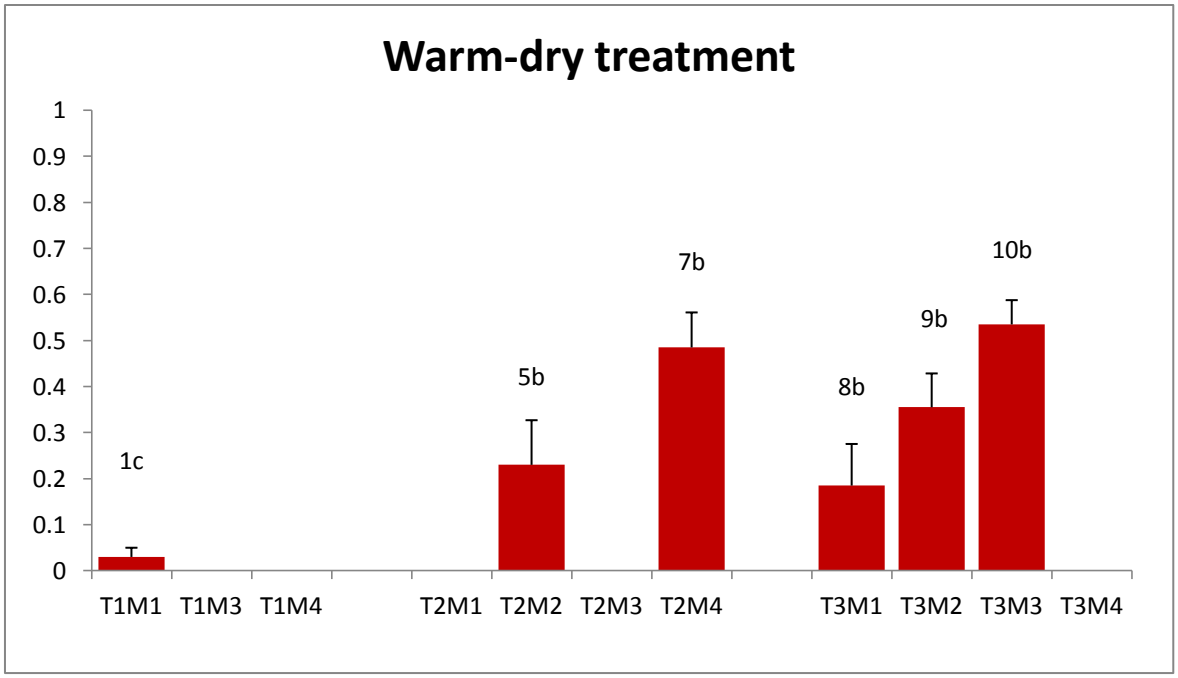

Figure 8 - Rate of germination in the warm-dry. Columns represent 11 original populations. Height of the column is the mean proportion + SE of germination of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population. Populations T1M3, T1M4, T2M1 and T2M3 did not have seeds in warm-dry and cold-dry treatments.

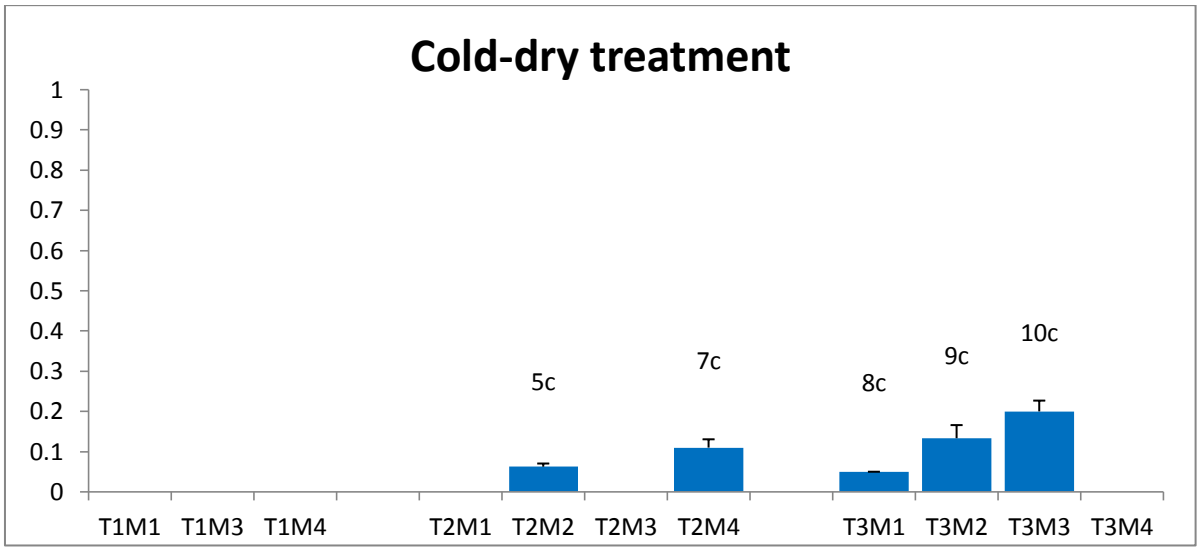

Figure 9 - Rate of germination in the cold-dry. Columns represent 11 original populations. Height of the column is the mean proportion + SE of germination of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population. Populations T1M3, T1M4, T2M1 and T2M3 did not have seeds in warm-dry and cold-dry treatments.

3.1.2 Germination index

GI measures the initial slope of the germination curve - greater GI indicates faster initial germination. Results are very similar to the rate of germination From the overall preview populations T1M4, T2M1, T2M3 germinated the fastest (Tab. 3). T2M4 germinated the fastest across all treatments (Fig. 10). Populations from T1 germinated as well as T3 populations in the warm-wet treatment (Fig. 11). The fastest initiation of germination was in the warm-wet treatment and the slowest was in cold-dry treatment. Fastest GI was 18.6 (warm-wet treatment) and the lowest GI was 0.10 (cold-dry treatment). In the dry treatments germination of the populations from the wet localities still germinated faster than those from dry localities (Fig. 13,14).

Temperature of the original locality had significant effect on initial germination (Tab. 2). Seeds from T2 germinated most. Also, temperature and moisture of the growth chamber had significant effect on seed germination. Seeds germinated better in warm-wet and cold-wet treatments. Interaction between moisture of the population and temperature of the growth chamber had significant effect on GI. Interaction between temperature of the population and

temperature of the growth chamber had significant effect on GI. Seeds originally from any temperature germinated better in warm treatments. Interaction between moisture and temperature of the growth chamber had also significant effect on GI (Tab. 2).

Population	overall	warm-wet	cold-wet	warm-dry	cold-dry
T1M1	8.01	18.63	13.37	0.05	0
T1M3	12.12	16.01	8.23	N/A	N/A
T1M4	13.48	15.25	11.71	N/A	N/A
T2M1	13.80	17.38	10.21	N/A	N/A
T2M2	5.94	15.35	8.07	0.29	0.07
T2M3	14.74	17.87	11.61	N/A	N/A
T2M4	8.76	18.62	15.28	1.00	0.27
T3M1	7.01	17.00	10.85	0.17	0.10
T3M2	7.97	16.85	14.33	0.61	0.14
T3M3	8.58	17.97	15.17	0.78	0.38
T3M4	7.85	18.50	12.88	0	0

Table 3 – Germination index across treatments - mean values of GI. Populations T1M3, T1M4, T2M1 and T2M3 did not have seeds in warm-dry and cold-dry treatments – marked as N/A. GI couldn't be calculated for populations where no seeds germinated = 0.

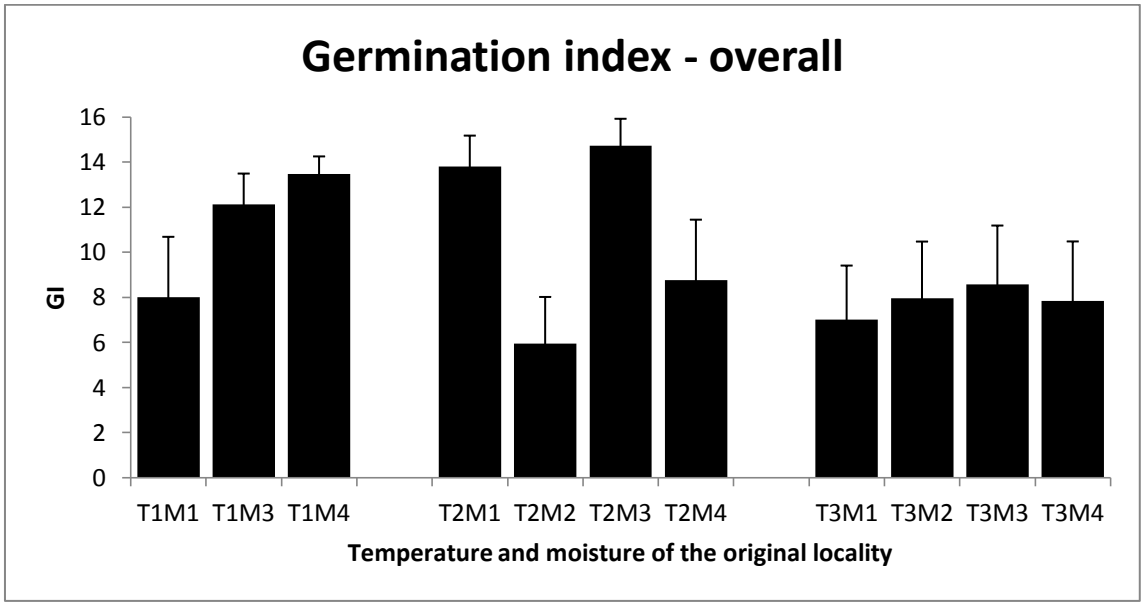

Figure 10 – Germination index - overall preview. Columns represent 11 original populations. Height of the column is the mean proportion + SE of germination of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest.

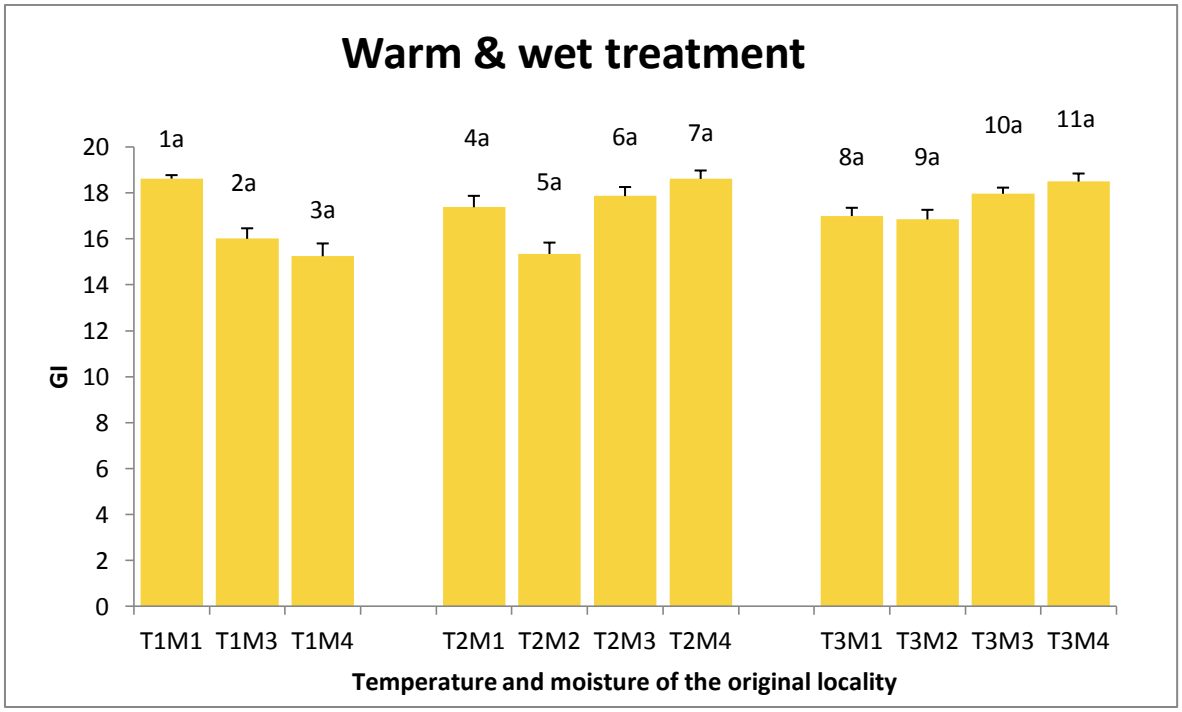

Figure 11 – Germination index in the warm-wet treatment. Columns represent 11 original populations. Height of the column is the mean proportion + SE of germination of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population.

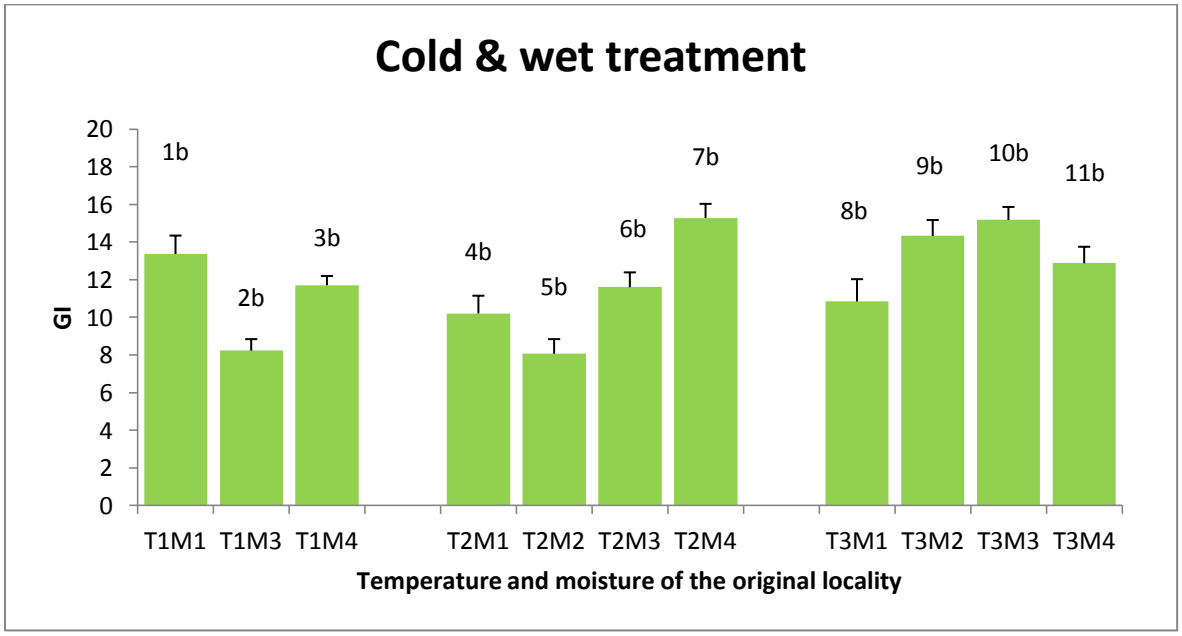

Figure 12 – Germination index in the cold-wet treatment. Columns represent 11 original populations. Height of the column is the mean proportion + SE of germination of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population.

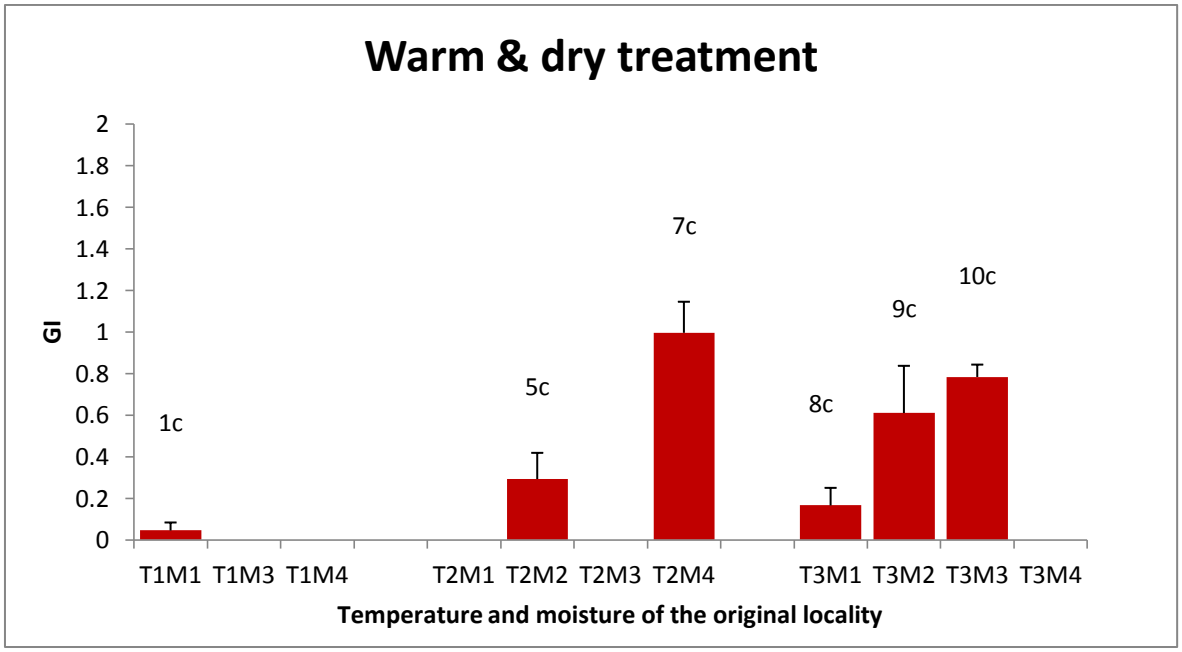

Figure 13 – Germination index in the warm-dry treatment. Columns represent 11 original populations. Height of the column is the mean proportion + SE of germination of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population. Populations T1M3, T1M4, T2M1 and T2M3 did not have seeds in warm-dry and cold-dry treatments.

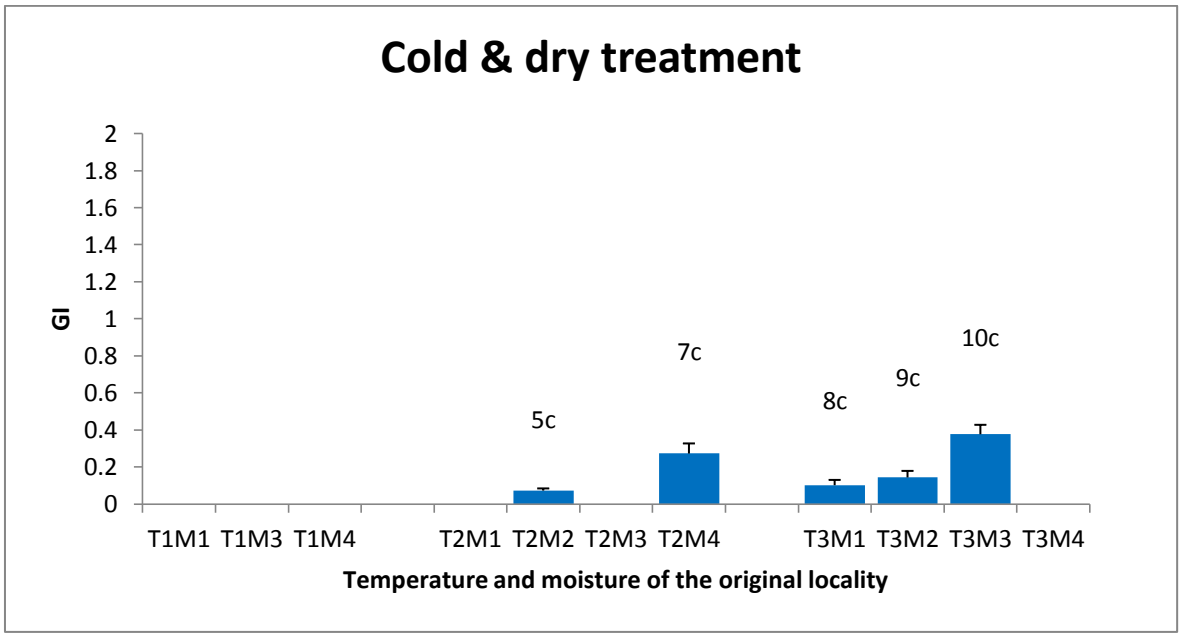

Figure 14 – Germination index in the cold-dry treatment. Columns represent 11 original populations. Height of the column is the mean proportion + SE of germination of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population. Populations T1M3, T1M4, T2M1 and T2M3 did not have seeds in warm-dry and cold-dry treatments.

3.1.3 Time to 50% germination events (T_{50})

T_{50} covers the complete germination curve and the lower T_{50}, the faster the germination. It is the time to when 50% of seeds germinated on the dish. Lower T50 indicates faster germination of seeds. Fastest germination was in T1 populations across all treatments (Tab. 4). Slowest germination was in T3 populations (Fig. 15). Fastest germination was in the warm-wet treatment T_{50} = 0.5 (Fig. 16). Highest T_{50} = 29 indicates slowest germination in cold-dry treatment (Fig.19).

T3 in the cold-wet treatment germinated fastest in cold-wet treatment despite the opposite of where seeds came from (Fig. 17). The same is true for T3M1 in the warm-dry treatment (Fig.18). Moisture and temperature of the original locality had significant effect on T_{50}. Moisture of the growth chamber had significant effect (Tab. 2). In the warm treatment seeds had lower T_{50}. Interaction between moisture and temperature of the original locality had significant effect on T_{50} (Tab. 2).

Population	overall	warm-wet	cold-wet	warm-dry	cold-dry
T1M1	1.09	0.56	1.03	0	0
T1M3	1.79	0.64	2.94	N/A	N/A
T1M4	1.03	0.79	1.28	N/A	N/A
T2M1	1.69	0.60	2.77	N/A	N/A
T2M2	4.89	0.66	1.99	18.21	0
T2M3	0.89	0.60	1.18	N/A	N/A
T2M4	4.57	0.56	0.73	11.38	9.75
T3M1	5.55	0.67	1.29	23.85	0
T3M2	7.20	0.63	0.92	17.99	21.25
T3M3	7.18	0.58	0.78	15.46	12.44
T3M4	0.85	0.57	1.13	0	0

Table 4 – T50 across treatments – mean values. T50 couldn't be counted in some populations in dry treatments. Populations T1M3, T1M4, T2M1 and T2M3 did not have seeds in warm-dry and cold-dry treatments – marked as N/A. T_{50} couldn't be counted from populations where no seeds germinated or the proportion of germinated seeds was too low (only 1 or 2 seeds) marked as 0 in this table.

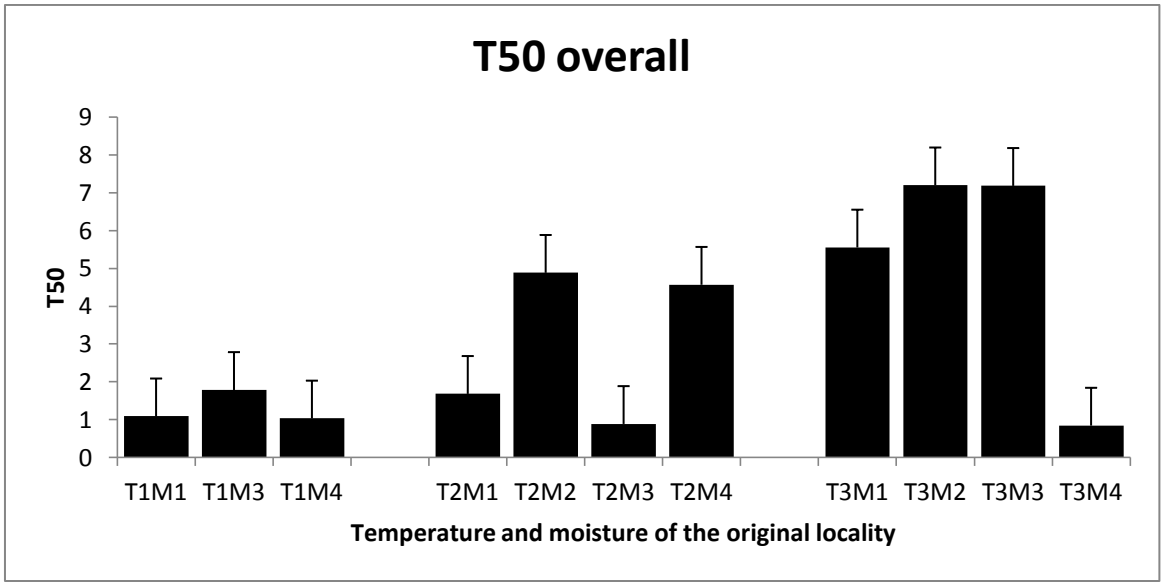

Figure 15 – T50 - overall preview. Columns represent 11 original populations. Height of the column is the mean proportion + SE of germination of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest.

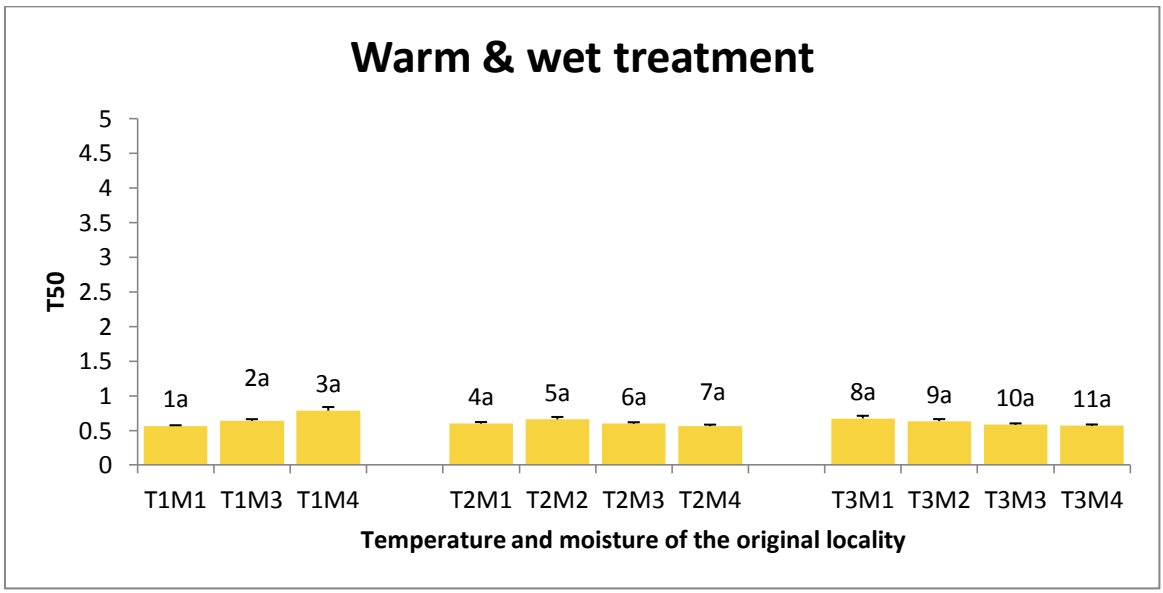

Figure 16 – T50 in the warm-wet treatment. Columns represent 11 original populations. Height of the column is the mean proportion + SE of germination of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population.

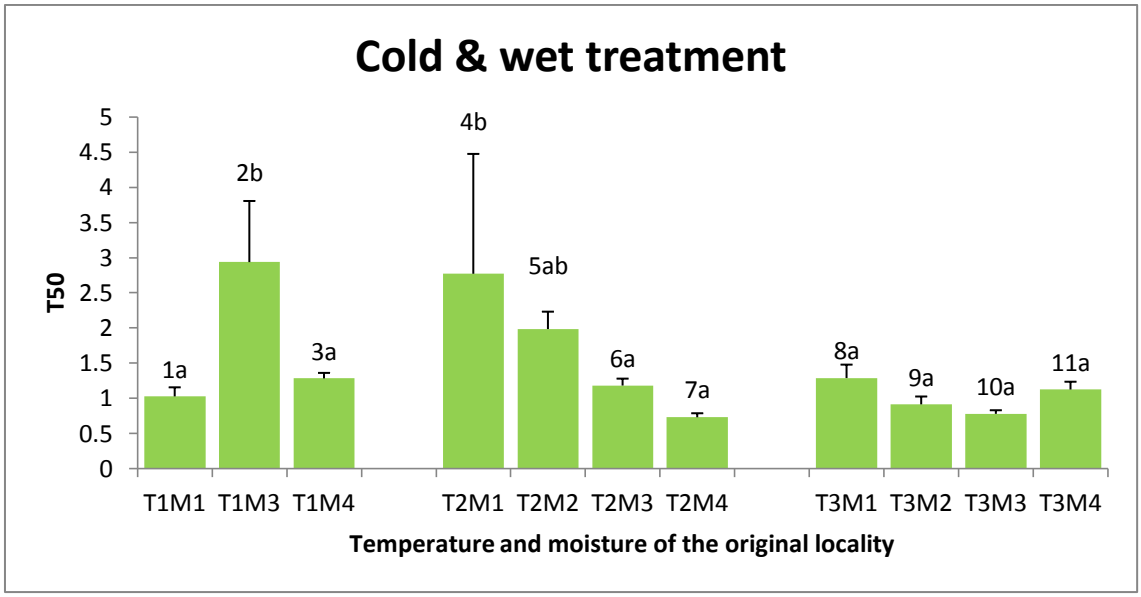

Figure 17 – T50 in the cold-wet treatment. Columns represent 11 original populations. Height of the column is the mean proportion + SE of germination of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population.

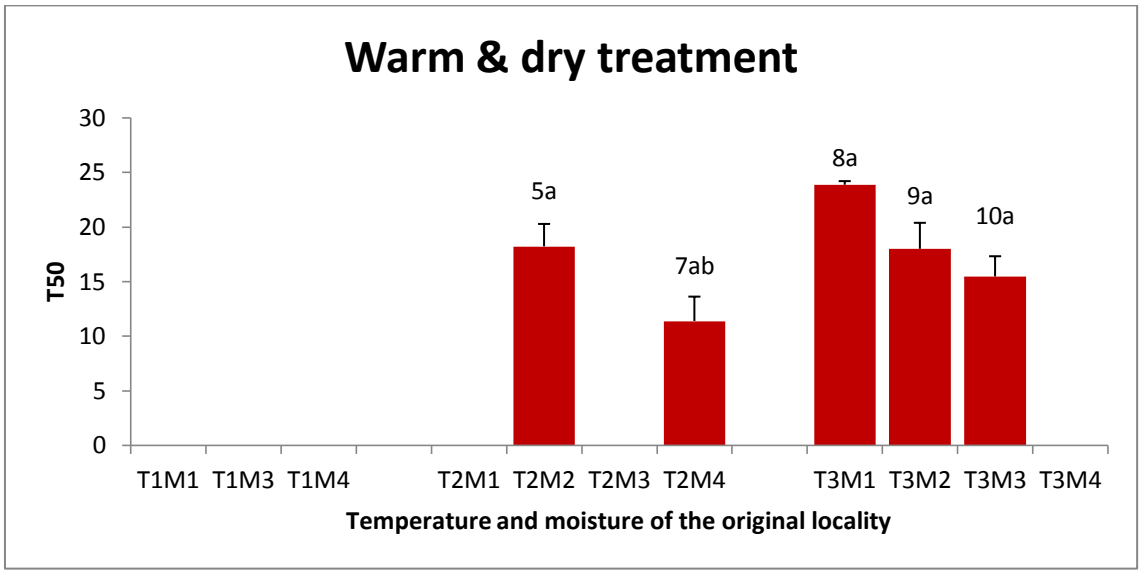

Figure 18 – T50 in the warm-dry treatment. Columns represent 11 original populations. Height of the column is the mean proportion + SE of germination of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population. Populations T1M3, T1M4, T2M1 and T2M3 did not have seeds in warm-dry and cold-dry treatments.

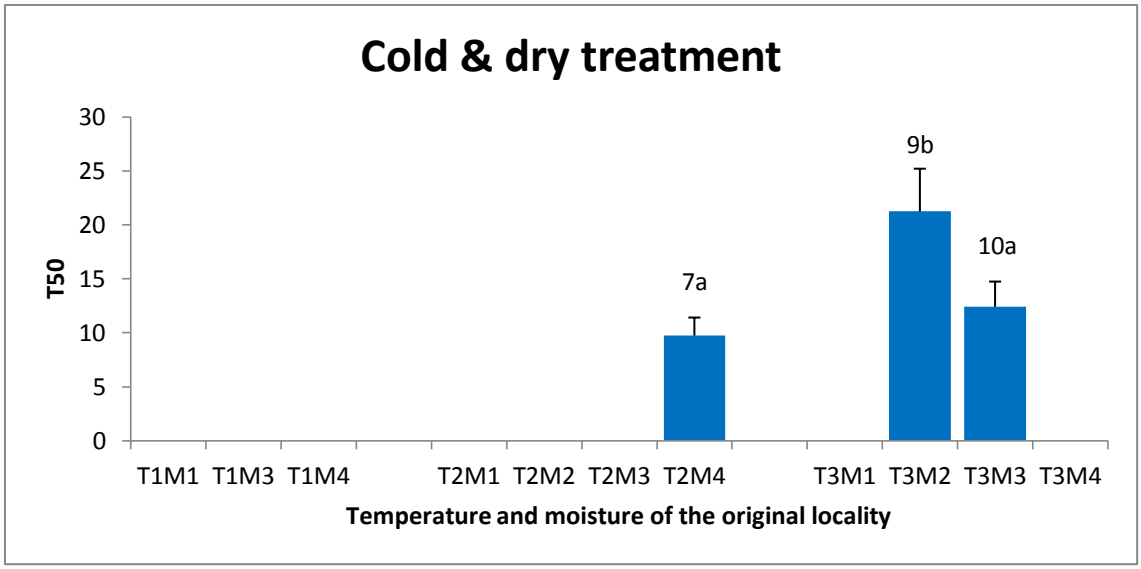

Figure 19 – T50 in the cold-dry treatment. Columns represent 11 original populations. Height of the column is the mean proportion + SE of germination of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population. Populations T1M3, T1M4, T2M1 and T2M3 did not have seeds in warm-dry and cold-dry treatments.

3.1.4 Proportion of viable seeds

Proportion of viable seeds was counted from all seeds that germinated and dormant seeds. Viability means that the seeds did not get moldy. Highest possible proportion is 1. Proportion of viable seeds varied across all treatments (Tab. 5). Highest viability was in the warm-wet and cold-dry treatment (Fig. 21, Fig. 24).

Temperature, moisture of the growth chamber and their interaction had the significant effect on viability of seeds. Seeds germinated better in the warm-wet treatment. Interaction between temperature and moisture of the original population had the significant effect on viability of seeds (Tab. 2).

Population	overall	warm-wet	cold-wet	warm-dry	cold-dry
T1M1	0.98	0.99	0.94	0.99	1.00
T1M3	0.88	0.91	0.86	N/A	N/A
T1M4	0.92	0.93	0.92	N/A	N/A
T2M1	0.93	0.95	0.92	N/A	N/A
T2M2	0.89	0.87	0.82	0.94	0.95
T2M3	0.92	0.97	0.88	N/A	N/A
T2M4	0.97	0.98	0.95	0.97	1.00
T3M1	0.95	0.97	0.90	0.95	1.00
T3M2	0.94	0.94	0.93	0.91	0.99
T3M3	0.95	0.97	0.94	0.92	0.98
T3M4	0.98	0.98	0.93	1.00	1.00

Table 5 – Viability across treatments – mean values, Populations T1M3, T1M4, T2M1 and T2M3 did not have seeds in warm-dry and cold-dry treatments – marked as N/A.

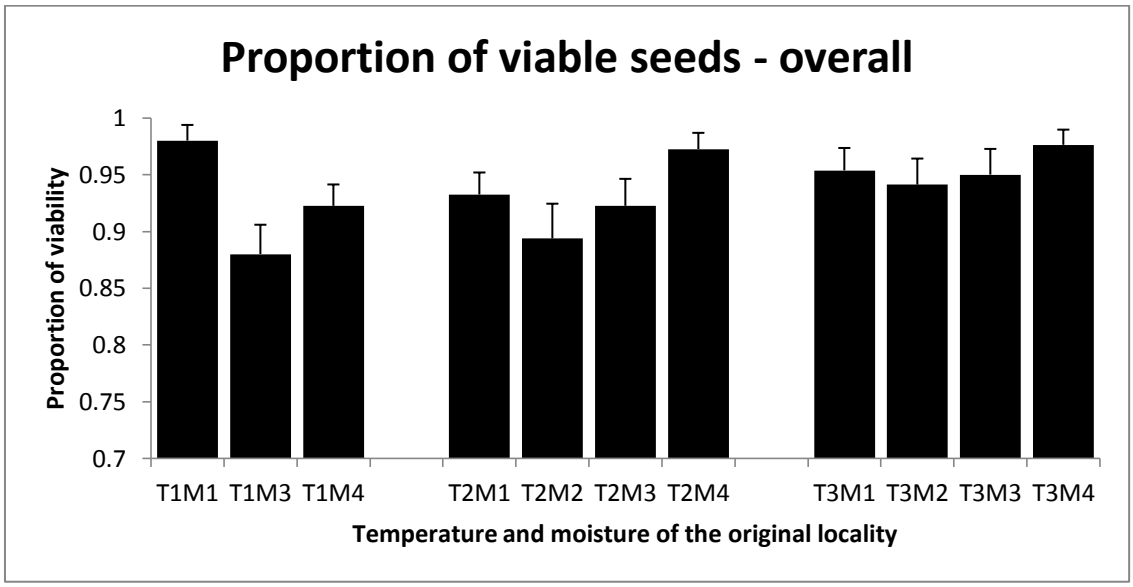

Figure 20 –Proportion of viable seeds- overall preview. Columns represent 11 original populations. Height of the column is the mean proportion + SE of germination of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest.

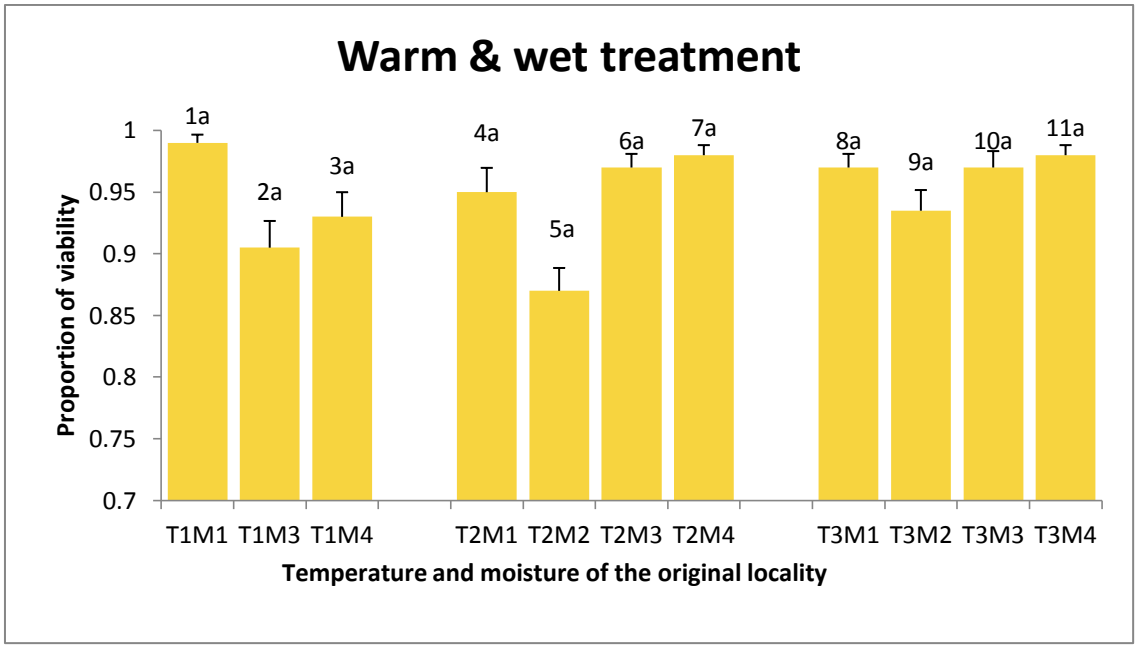

Figure 21 –Proportion of viable seeds in the warm-wet treatment. Columns represent 11 original populations. Height of the column is the mean proportion + SE of germination of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population.

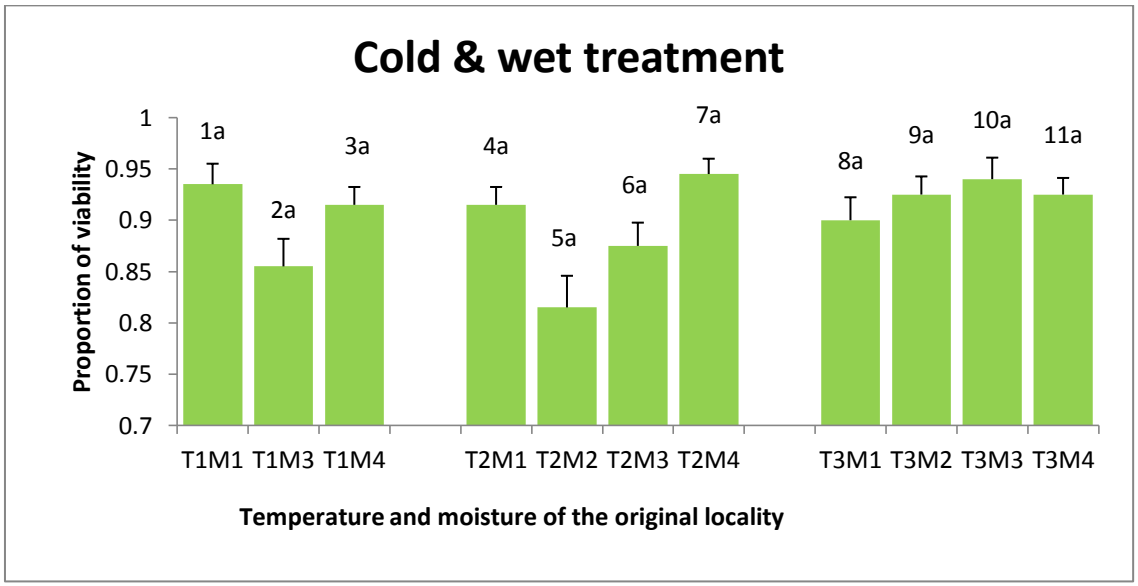

Figure 22 –Proportion of viable seeds in the cold-wet treatment. Columns represent 11 original populations. Height of the column is the mean proportion + SE of germination of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population.

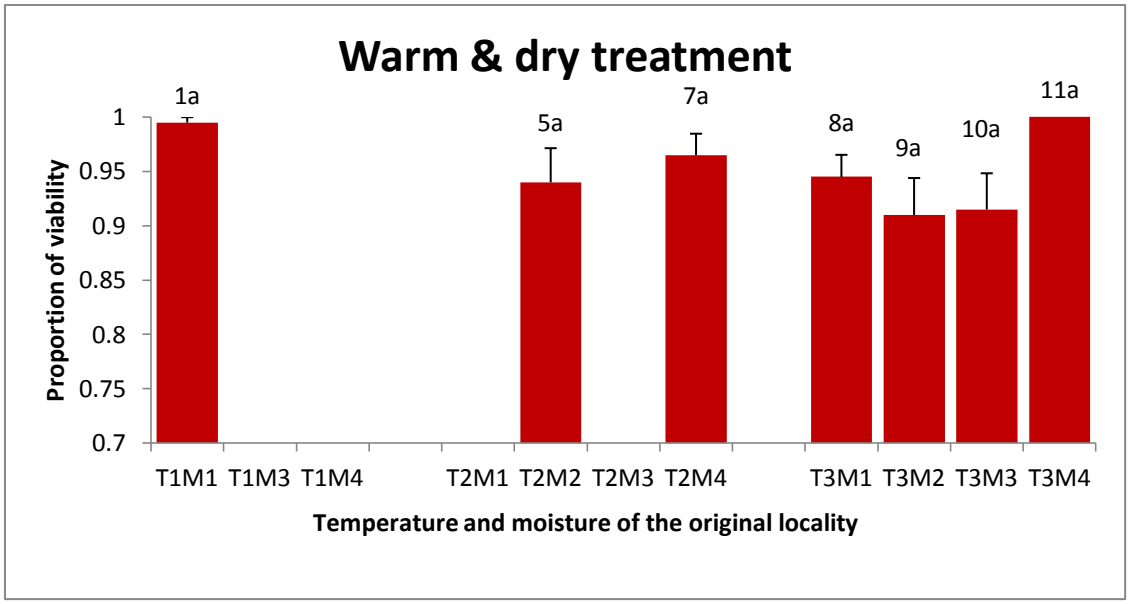

Figure 23 –Proportion of viable seeds in the warm-dry treatment. Columns represent 11 original populations. Height of the column is the mean proportion + SE of germination of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population. Populations T1M3, T1M4, T2M1 and T2M3 did not have seeds in warm-dry and cold-dry treatments.

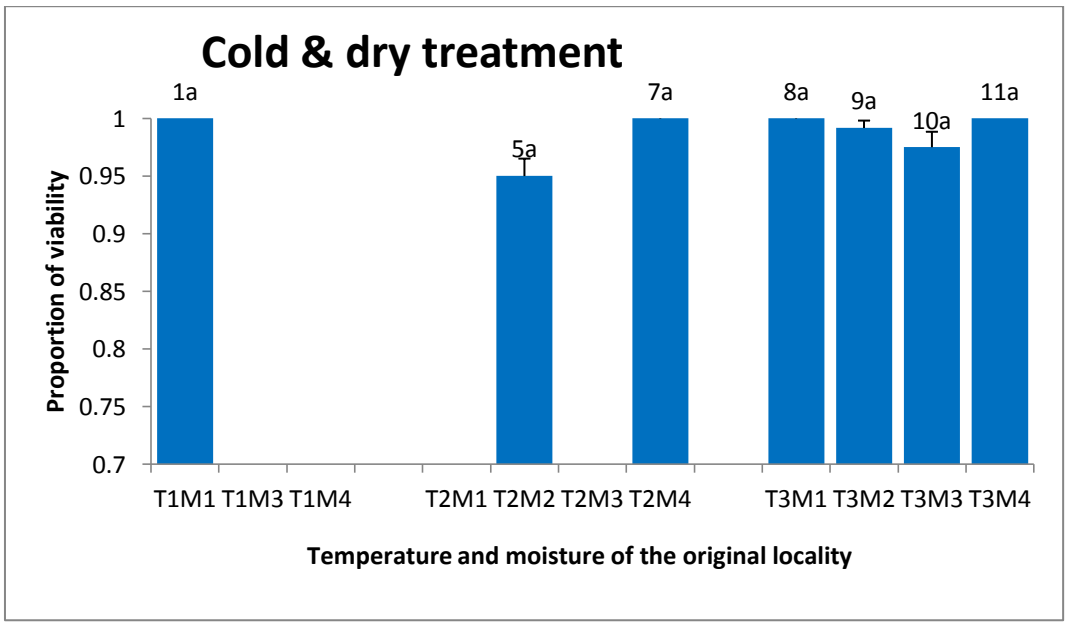

Figure 24 –Proportion of viable seeds in the cold-dry treatment. Columns represent 11 original populations. Height of the column is the mean proportion + SE of germination of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population. Populations T1M3, T1M4, T2M1 and T2M3 did not have seeds in warm-dry and cold-dry treatments.

3.1.5 Proportion of dormant seeds

Dormant seeds in this study are those that did not germinate before termination of the germination part. Viability was proven by germination in optimal conditions or by colouring with tetrazolium chloride. Highest possible proportion of dormancy is 1 and lowest is 0 (Tab.6).

There were no dormant seeds in the warm-wet treatment. All of the seeds in this treatment germinated or were mouldy and were removed. In the cold-wet treatment there were very little of dormant seeds (Fig. 26). In the cold dry treatment was the proportion of dormancy the highest (Fig. 28). Seeds from this treatment did not germinate and did not get mouldy. Temperature, moisture of the growth chamber and their interaction had the significant effect on dormancy of seeds (Tab. 2). The most proportion of dormant seeds was in the cold-dry treatment. Interaction between temperature and moisture of the original population had the significant effect on dormancy of seeds (Tab. 2).

Population	overall	warm-wet	cold-wet	warm-dry	cold-dry
T1M1	0.49		0.01	0.97	1.00
T1M3	0.03		0.05	N/A	N/A
T1M4	0.00		0.00	N/A	N/A
T2M1	0.00		0.01	N/A	N/A
T2M2	0.42		0.00	0.72	0.94
T2M3	0.00		0.00	N/A	N/A
T2M4	0.36		0.02	0.49	0.94
T3M1	0.45		0.02	0.79	0.99
T3M2	0.38		0.00	0.59	0.92
T3M3	0.30		0.00	0.41	0.79
T3M4	0.50		0.00	0.50	1.00

Table 6 – Proportion of dormancy across treatments – mean values. Populations T1M3, T1M4, T2M1 and T2M3 did not have seeds in warm-dry and cold-dry treatments. Populations T1M3, T1M4, T2M1 and T2M3 did not have seeds in warm-dry and cold-dry treatments – marked as N/A.

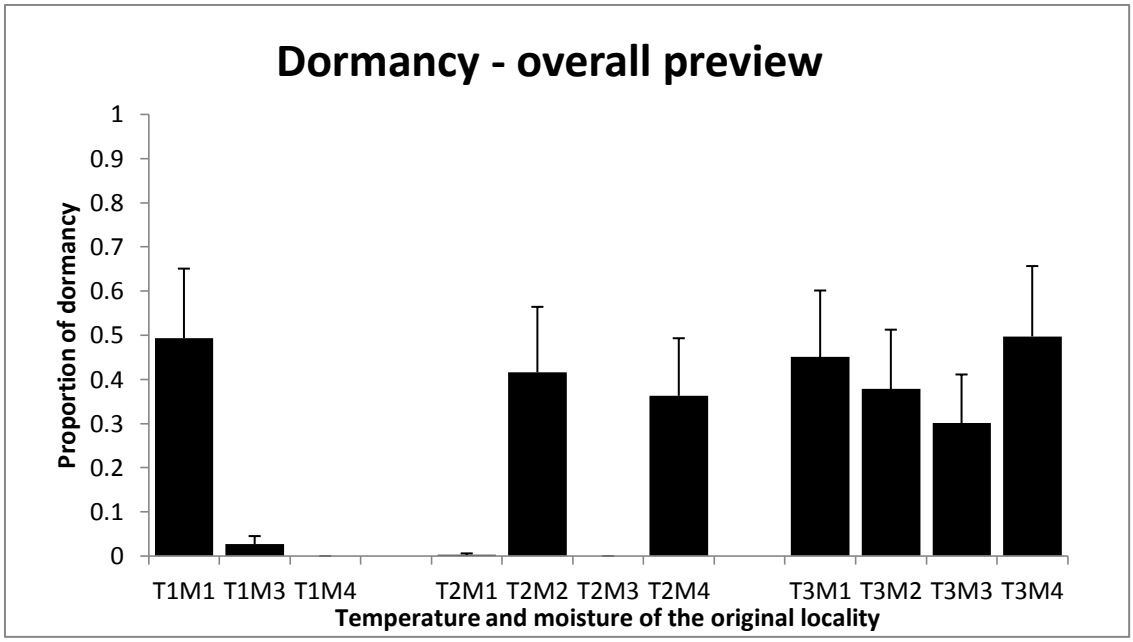

Figure 25 –Proportion of dormant seeds – overall preview. Columns represent 11 original populations. Height of the column is the mean proportion + SE of germination of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest.

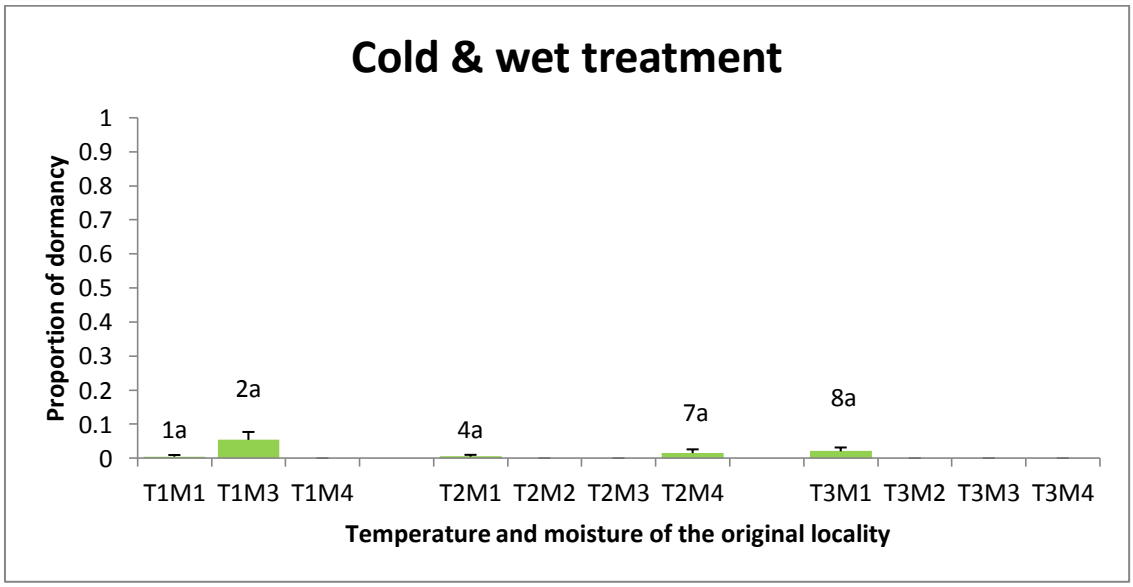

Figure 26 –Proportion of dormant seeds in the cold-wet treatment. Columns represent 11 original populations. Height of the column is the mean proportion + SE of germination of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population.

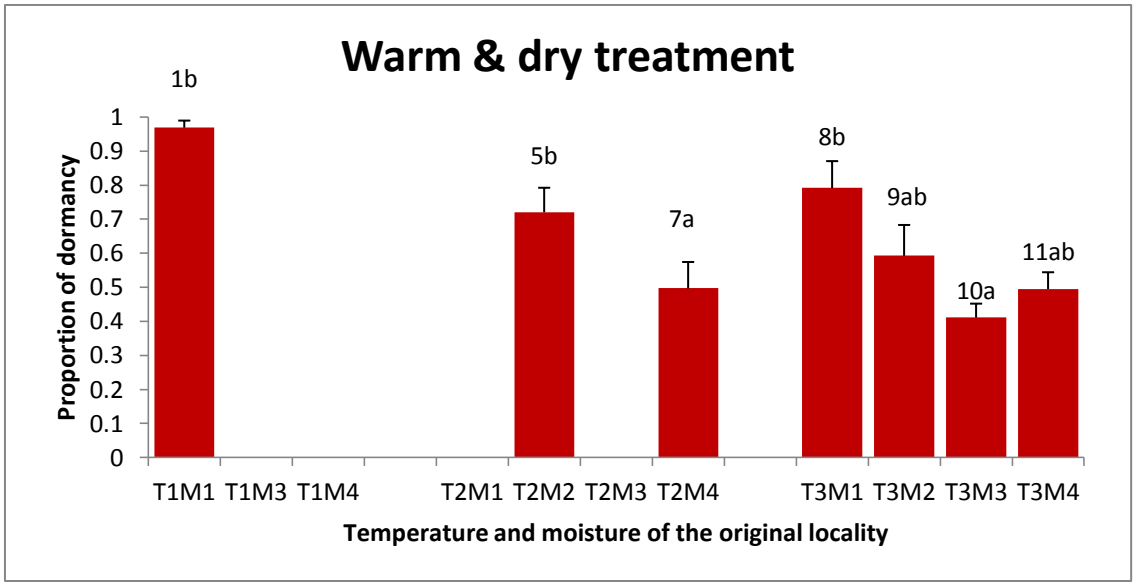

Figure 27 –Proportion of dormant seeds in the warm-dry treatment. Columns represent 11 original populations. Height of the column is the mean proportion + SE of germination of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population. Populations T1M3, T1M4, T2M1 and T2M3 did not have seeds in warm-dry and cold-dry treatments.

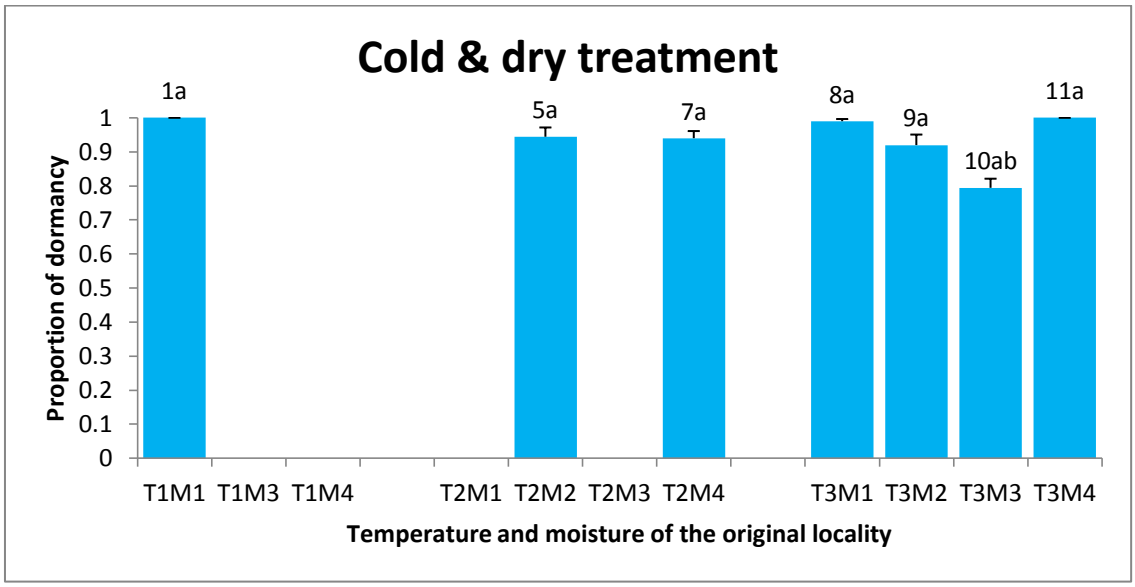

Figure 28 –Proportion of dormant seeds in the cold-dry treatment. Columns represent 11 original populations. Height of the column is the mean proportion + SE of germination of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population. Populations T1M3, T1M4, T2M1 and T2M3 did not have seeds in warm-dry and cold-dry treatments.

3.2 Growth of plants

3.2.1 Number of ramets in the pot

Highest number of ramets were in T1M4, T2M4, T3M3 (Tab. 7). Highest mean of the ramets was in T2 populations = 66.5 in overall preview (Fig.29). Warm-wet treatment was second in mean number of ramets in comparison to other treatments. Cold-dry treatment had the highest mean number of ramets (mean = 72.2) and also highest number of ramets was from T3M3 population with 208 ramets (Fig. 33). Lowest number of ramets was from T2M2 in cold-dry treatment.

Moisture of the growth chamber had significant effect on number of ramets in the pot (Tab. 8). In the dry treatments if the seeds germinated, ramets were more dense. Moisture and temperature of the original locality interacted with moisture of the growth chamber. Ramets from more moist localities and any temperature were more dense.

Population	overall	warm-wet	cold-wet	warm-dry	cold-dry
T1M1	51.61	56.70	62.00	0	0
T1M3	63.30	59.90	66.70	N/A	N/A
T1M4	79.90	85.20	74.60	N/A	N/A
T2M1	70.45	73.60	67.30	N/A	N/A
T2M2	48.41	47.60	64.70	43.60	15.75
T2M3	69.05	75.10	63.00	N/A	N/A
T2M4	75.40	68.00	57.40	93.70	89.60
T3M1	65.81	75.60	82.40	20.40	47.50
T3M2	52.75	71.70	69.40	34.10	24.50
T3M3	83.65	68.70	70.20	76.00	119.70
T3M4	58.81	65.00	58.50	0	0

Table 7 – Ramets across treatments – mean values. Populations T1M3, T1M4, T2M1 and T2M3 did not have seeds in warm-dry and cold-dry treatments – marked as N/A.

Df error = 276	RAMETS		HEIGHT		WEIGHT	
	Deviance	p	F value	p	F value	p
M.pop	22.49	**0.002**	0.796	0.373	30.781	**<0.001**
T.pop	2.20	0.341	1.394	0.239	0.335	0.563
M.clim	10.37	**0.039**	6.484	**0.011**	10.397	**0.001**
T.clim	1.81	0.388	57.690	**<0.001**	28.146	**<0.001**
M.pop:T.pop	4.97	0.152	7.649	**0.006**	0.473	0.492
M.pop:M.clim	162.46	**<0.001**	11.936	**0.001**	64.099	**<0.001**
T.pop:M.clim	25.38	**0.001**	7.081	**0.008**	4.899	**0.028**
M.pop:T.clim	1.57	0.420	0.230	0.632	0.258	0.612
T.pop:T.clim	0.27	0.738	0.562	0.454	0.703	0.403
M.clim:T.clim	1.64	0.411	46.183	**<0.001**	5.568	**0.019**
M.pop:T.pop:M.clim	4.55	0.171	2.534	0.113	2.432	0.120
M.pop:T.pop:T.clim	1.03	0.515	2.713	0.101	0.412	0.522
M.pop:M.clim:T.clim	8.71	0.058	3.380	0.067	4.970	**0.027**
T.pop:M.clim:T.clim	0.25	0.748	0.174	0.677	0.184	0.668
M.pop:T.pop:M.clim:T.clim	2.46	0.314	0.385	0.536	0.344	0.558

Table 8 – Results - Significant results are bold. Df – degrees of freedom. M – moisture, T – temperature, pop – original population, clim – climate of growth chamber

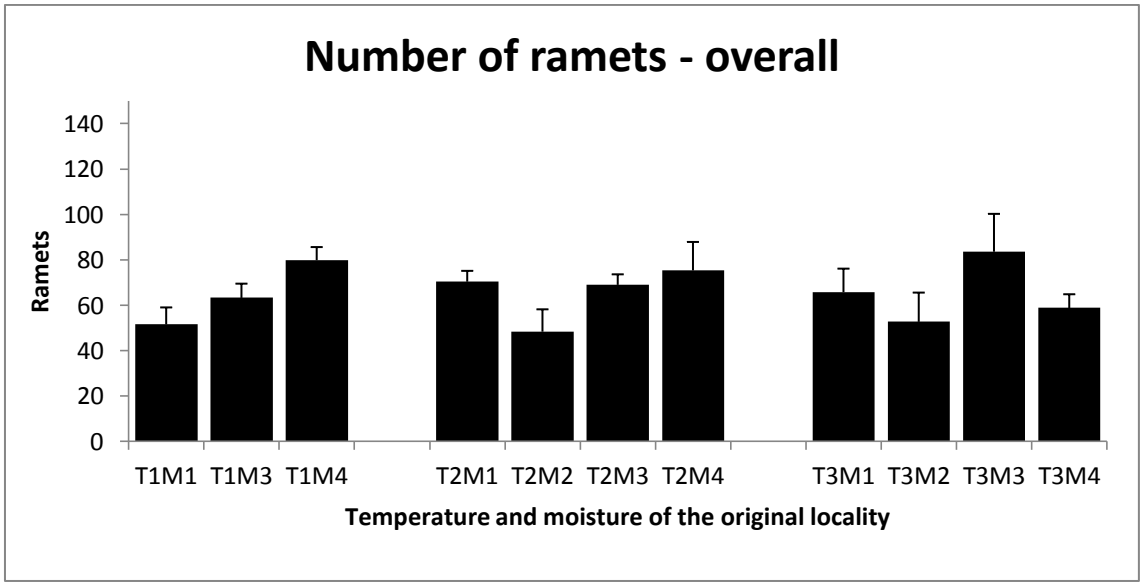

Figure 29 – Number of ramets - overall preview. Columns represent 11 original populations. Height of the column is the mean proportion + SE of ramets from one pot in the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest.

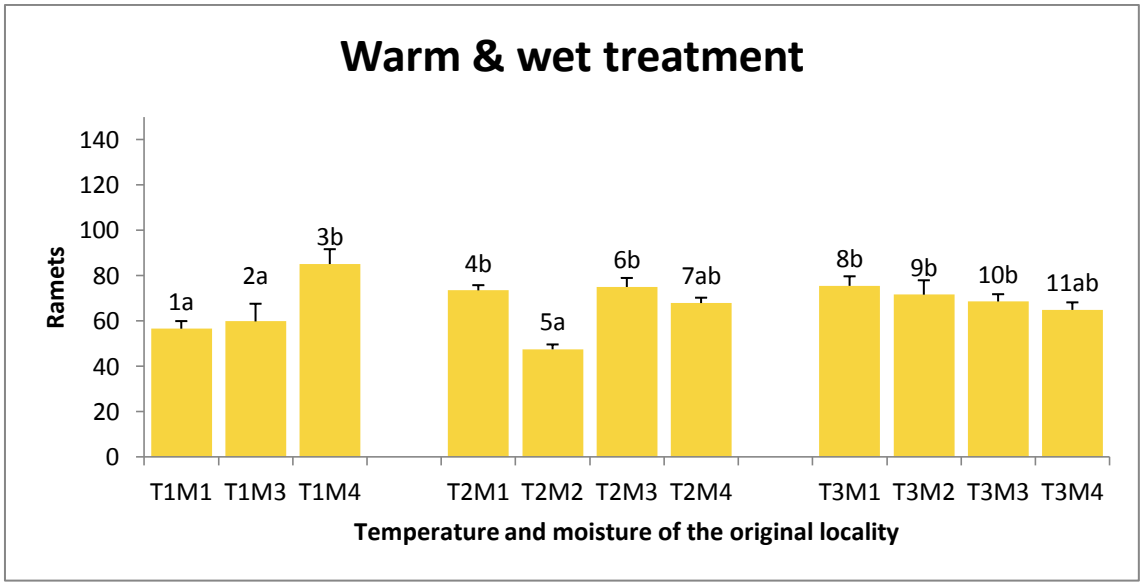

Figure 30 – Number of ramets in the warm-wet treatment. Columns represent 11 original populations. Height of the column is the mean proportion + SE of ramets from one pot in the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population.

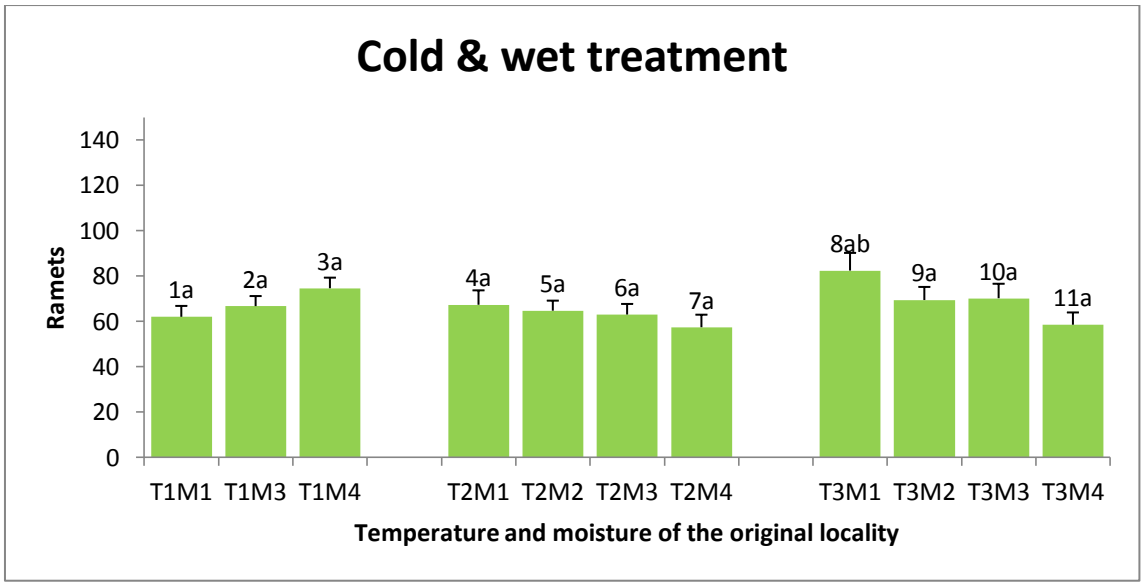

Figure 31 – Number of ramets in the cold-wet treatment. Columns represent 11 original populations. Height of the column is the mean proportion + SE of ramets from one pot in the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population.

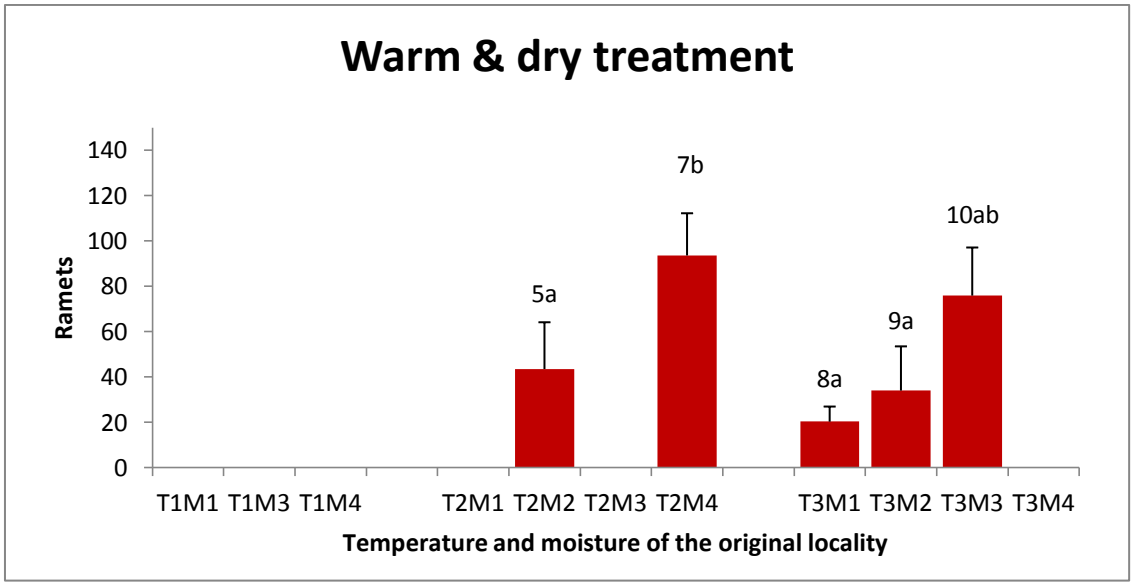

Figure 32 – Number of ramets in the warm-dry treatment. Columns represent 11 original populations. Height of the column is the mean proportion + SE of ramets from one pot in the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population. Populations T1M3, T1M4, T2M1 and T2M3 did not have seeds in warm-dry and cold-dry treatments.

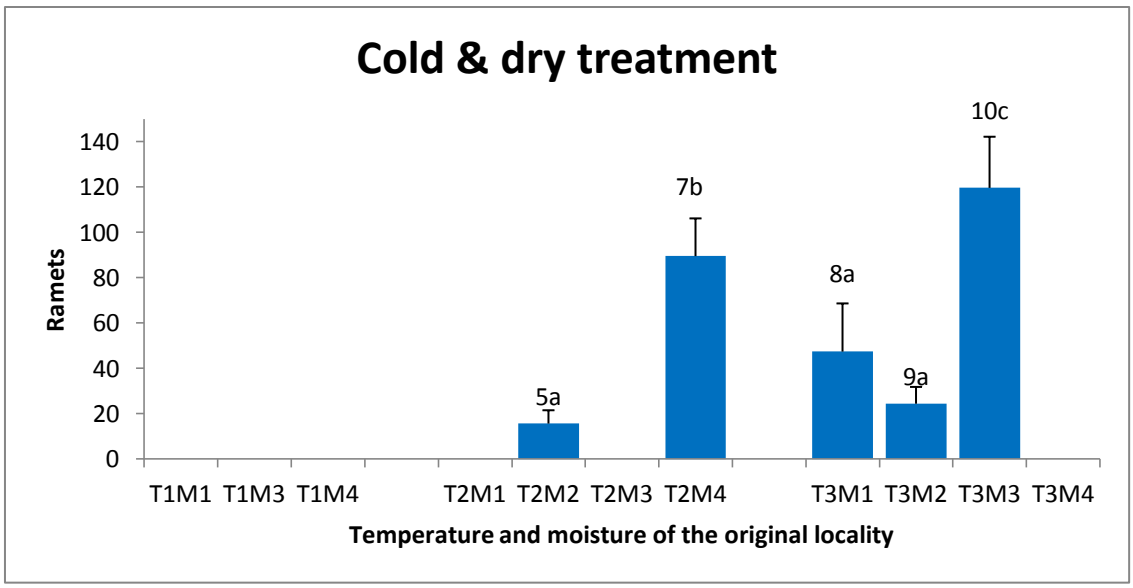

Figure 33 – Number of ramets in the cold-dry treatment. Columns represent 11 original populations. Height of the column is the mean proportion + SE of ramets from one pot in the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population. Populations T1M3, T1M4, T2M1 and T2M3 did not have seeds in warm-dry and cold-dry treatments.

3.2.2 Height of plants

Height of plants was quite even in the overall comparison of populations ranging from 16.5 cm to 21.3 cm (Fig 34., Tab. 9). Highest plants were from the warm-wet treatment (Fig. 35). T2M2 and T3M2 were the highest – mean height of 25.6 cm and 25.5 cm. Smallest plants were measured in the warm-dry treatment T3M1 population – mean height of 5.6 cm. T1M1 plants were comparably tall as T3M4 in the warm-wet treatment.

Effects of moisture and temperature of the original locality had significant effect on height of plants (Tab. 8). Tallest plants were in the warm-wet treatment. Moisture and temperature of the original locality interacted with moisture of the growth chamber. Moisture and temperature of the growth chamber interacted with each other.

Population	overall	warm-wet	cold-wet	warm-dry	cold-dry
T1M1	19.20	22.20	16.20	0	0
T1M3	20.35	19.55	19.12	N/A	N/A
T1M4	18.28	19.85	16.70	N/A	N/A
T2M1	18.98	20.55	17.40	N/A	N/A
T2M2	21.05	25.60	19.53	10.80	10.50
T2M3	18.78	21.70	15.85	N/A	N/A
T2M4	18.58	21.30	14.90	17.22	15.54
T3M1	16.52	20.10	15.00	5.60	8.75
T3M2	20.70	25.45	19.10	6.10	19.08
T3M3	21.34	22.90	19.95	14.10	19.87
T3M4	20.03	23.90	16.15	0	0

Table 9 – Height of plants across treatments – mean values. Populations T1M3, T1M4, T2M1 and T2M3 did not have seeds in warm-dry and cold-dry treatments. Populations T1M3, T1M4, T2M1 and T2M3 did not have seeds in warm-dry and cold-dry treatments – marked as N/A.

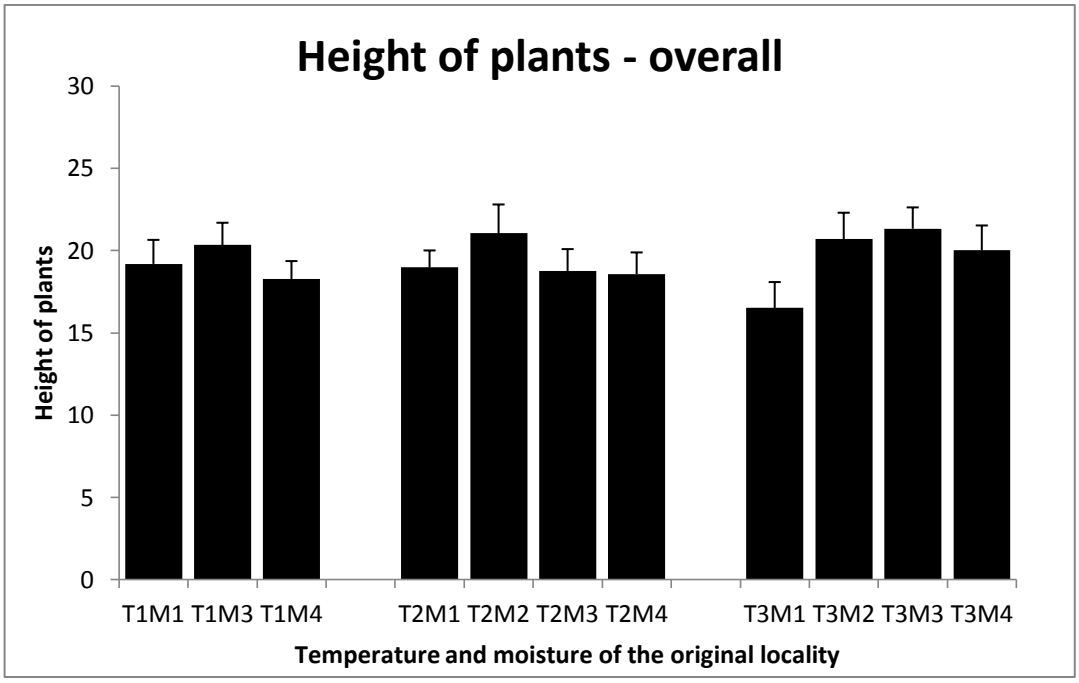

Figure 34 – Height of ramets - overall preview. Columns represent 11 original populations. Height of the column is the mean proportion + SE of height of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population.

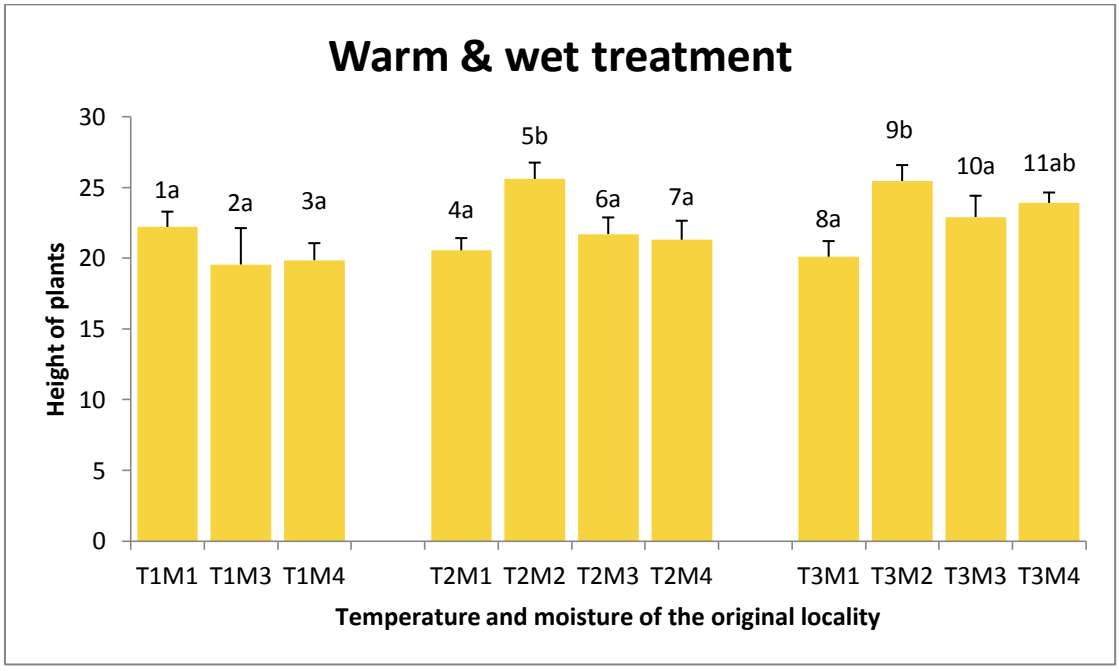

Figure 35 – Height of ramets in the warm-wet treatment. Columns represent 11 original populations. Height of the column is the mean proportion + SE of height of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population

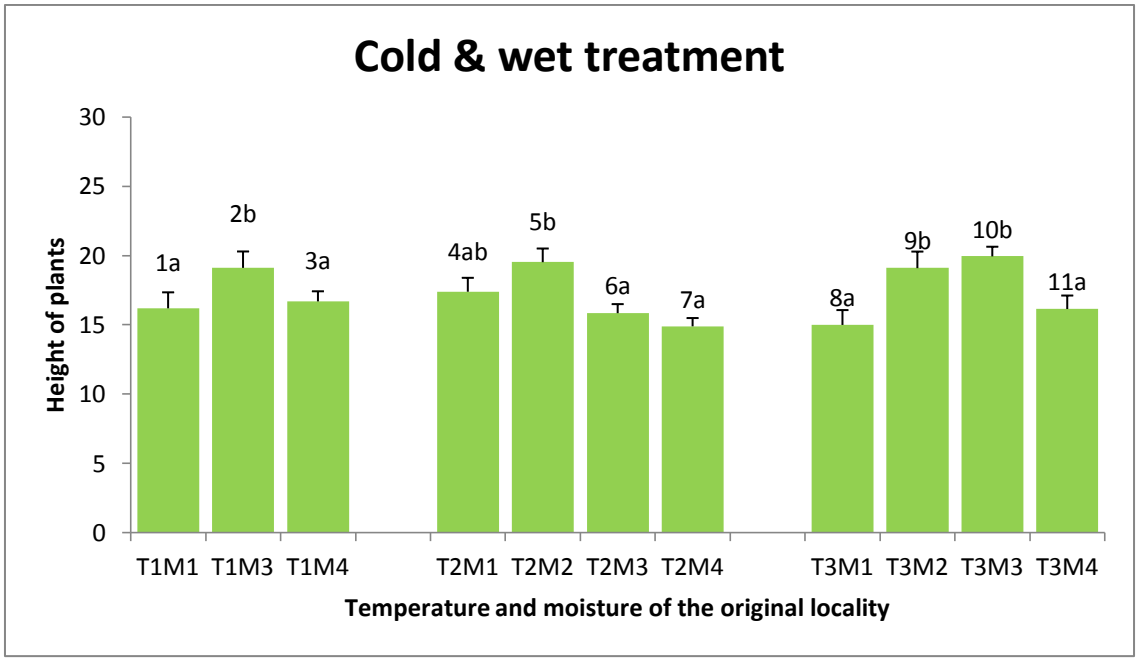

Figure 36 – Height of ramets in the cold-wet treatment. Columns represent 11 original populations. Height of the column is the mean proportion + SE of height of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population. Populations T1M3, T1M4, T2M1 and T2M3 did not have seeds in warm-dry and cold-dry treatments.

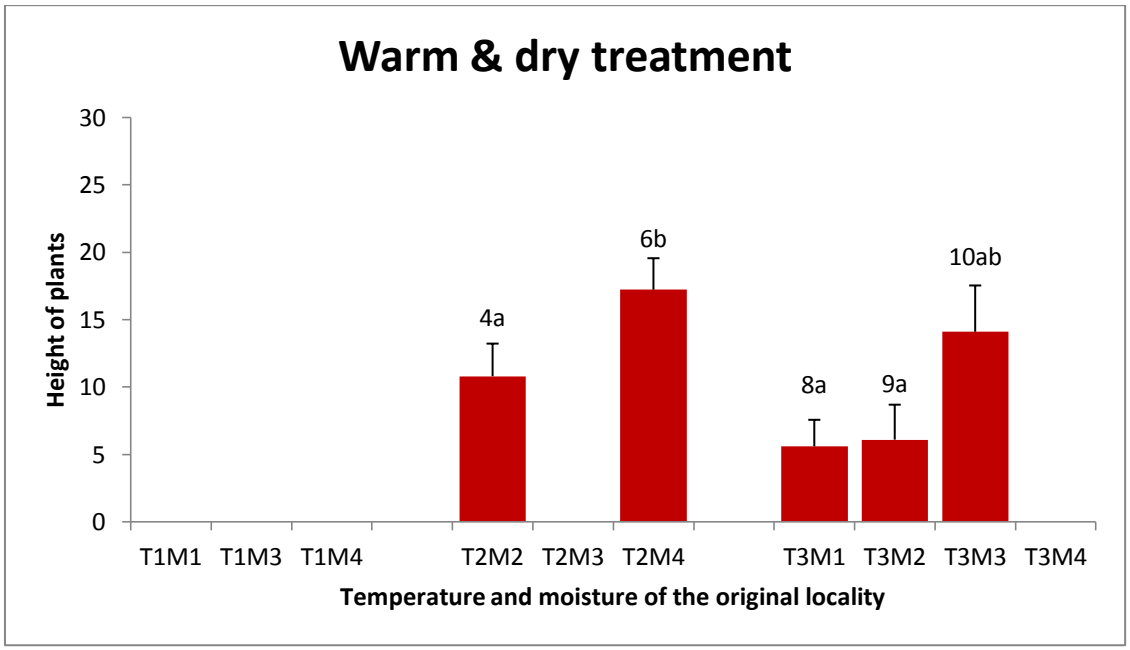

Figure 37 – Height of ramets in the warm-dry treatment. Columns represent 11 original populations. Height of the column is the mean proportion + SE of height of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population. Populations T1M3, T1M4, T2M1 and T2M3 did not have seeds in warm-dry and cold-dry treatments.

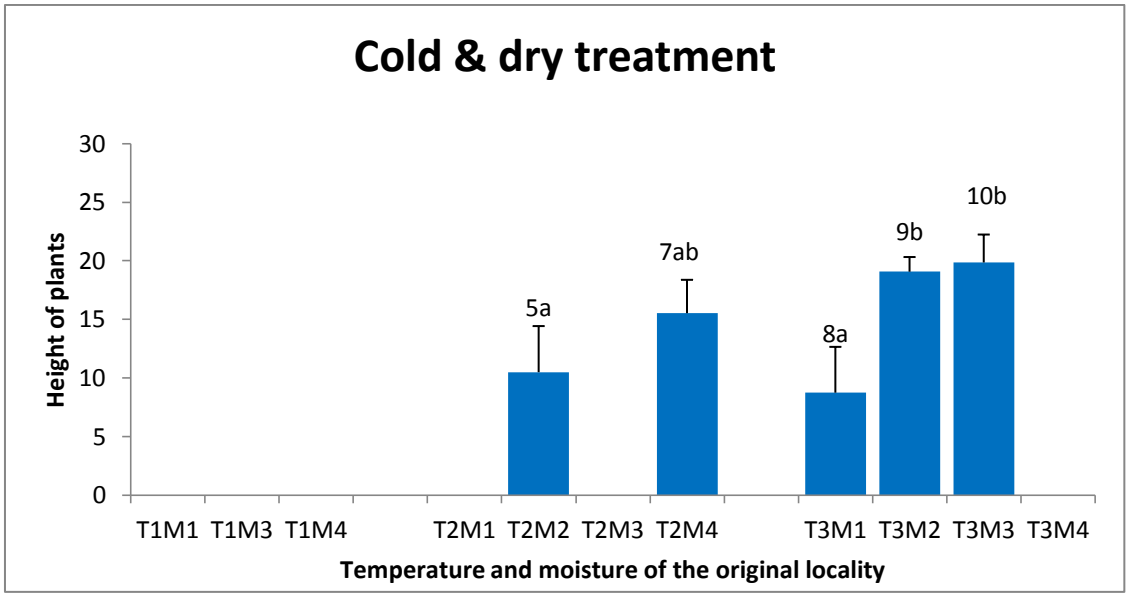

Figure 38 – Height of ramets in the cold-dry treatment. Columns represent 11 original populations. Height of the column is the mean proportion + SE of height of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population. Populations T1M3, T1M4, T2M1 and T2M3 did not have seeds in warm-dry and cold-dry treatments.

3.2.3 Weight of biomass

Biomass, which was cut 18 weeks after the start of germination part, was kept. The dried biomass was weighted in grams. The most biomass produced was in populations T2M4 and T3M3 in the overall comparison of populations (Fig. 39). More biomass was produced in the dry treatments than in wet treatments. T2M4 and T3M3 produced the most biomass in the cold-dry treatment despite being from the opposite origin. In the warm-wet treatment the mean weight of biomass from all populations was the lowest. (Tab. 10)

Moisture of the original population had significant effect on weight of biomass. Seeds from M3, M4 produced more biomass. Also, moisture and temperature of the growth chamber had significant effect on weight of biomass (Tab. 8). In the warm-dry, cold-dry treatments if the seeds germinated, they also produced more biomass. Moisture and temperature of the original locality had significant interaction with moisture of the growth chamber. Moisture and temperature of the growth chamber interacted with each other.

Population	overall	warm-wet	cold-wet	warm-dry	cold-dry
T1M1	1.42	1.097	1.75	0	0
T1M3	2.11	1.456	2.44	N/A	N/A
T1M4	1.92	1.824	2.01	N/A	N/A
T2M1	1.64	1.23	2.06	N/A	N/A
T2M2	1.66	1.32	2.13	1.83	0.67
T2M3	1.98	1.91	2.06	N/A	N/A
T2M4	2.43	1.27	2.02	2.65	4.34
T3M1	1.40	1.20	1.73	0.49	2.81
T3M2	1.74	1.49	2.44	2.39	0.63
T3M3	2.60	1.48	1.93	3.27	4.05
T3M4	1.78	1.43	1.95	0	0

Table 10 – Weight of plants across treatments – mean values. Populations T1M3, T1M4, T2M1 and T2M3 did not have seeds in warm-dry and cold-dry treatments – marked as N/A.

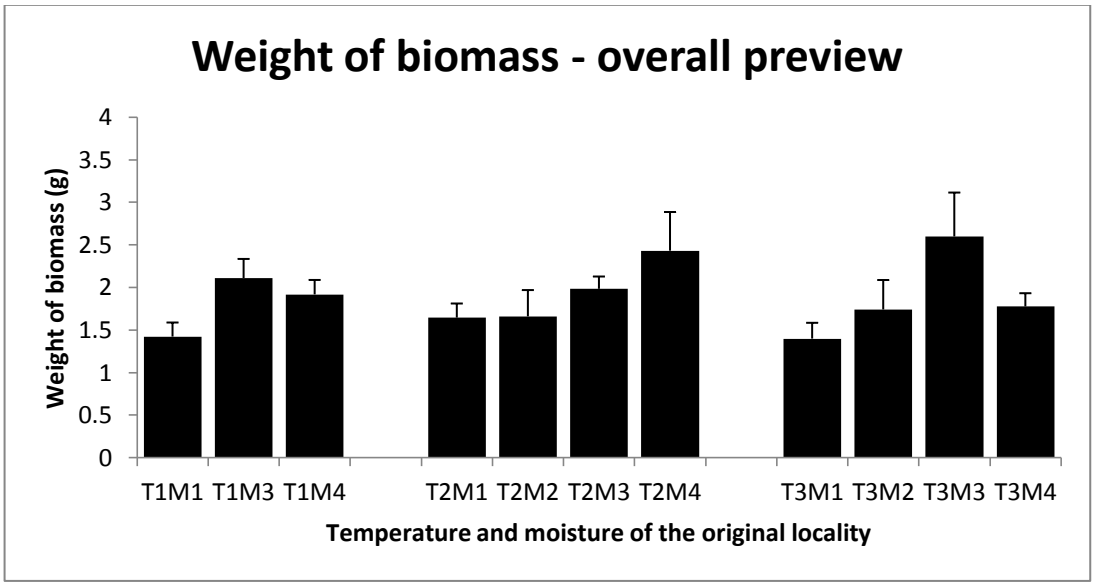

Figure 39 – Weight of biomass – overall preview. Columns represent 11 original populations. Height of the column is the mean proportion + SE of biomass of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest.

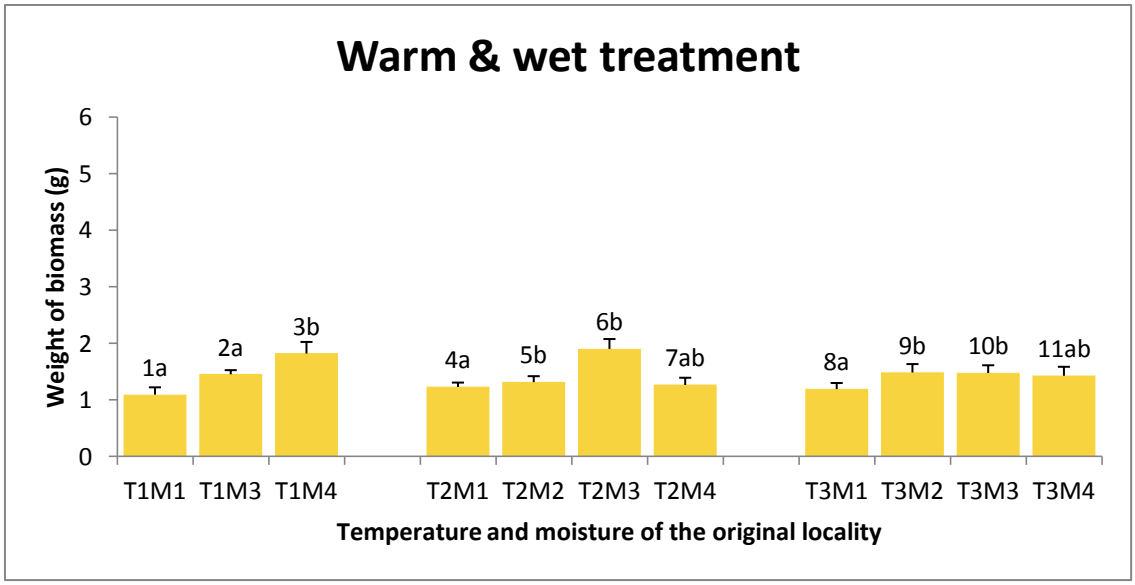

Figure 40 – Weight of biomass in the warm-wet treatment. Columns represent 11 original populations. Height of the column is the mean proportion + SE of biomass of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population

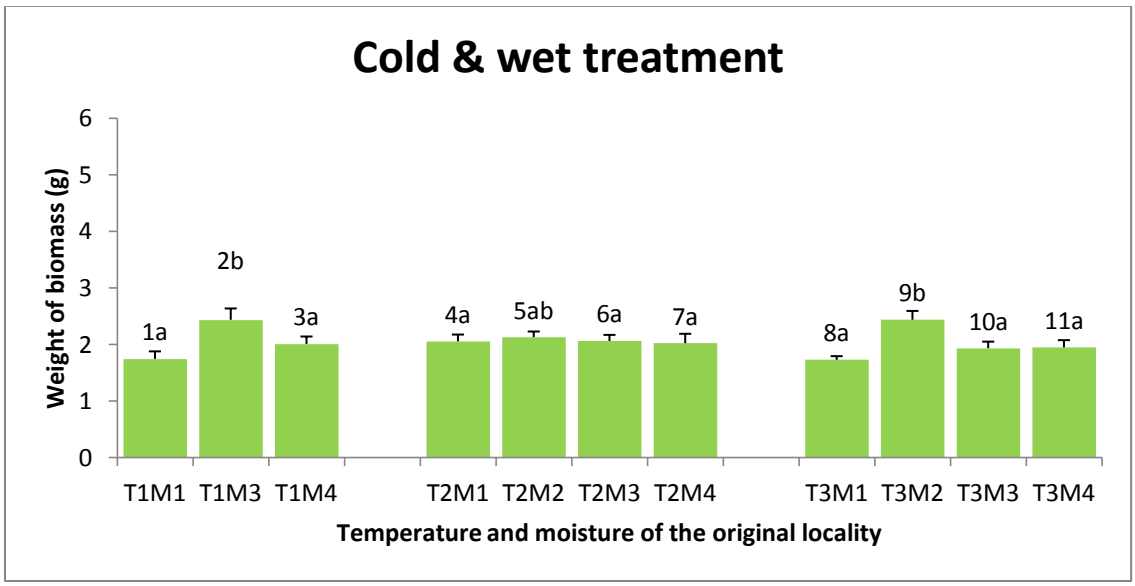

Figure 41 – Weight of biomass in the cold-wet treatment. Columns represent 11 original populations. Height of the column is the mean proportion + SE of biomass of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population

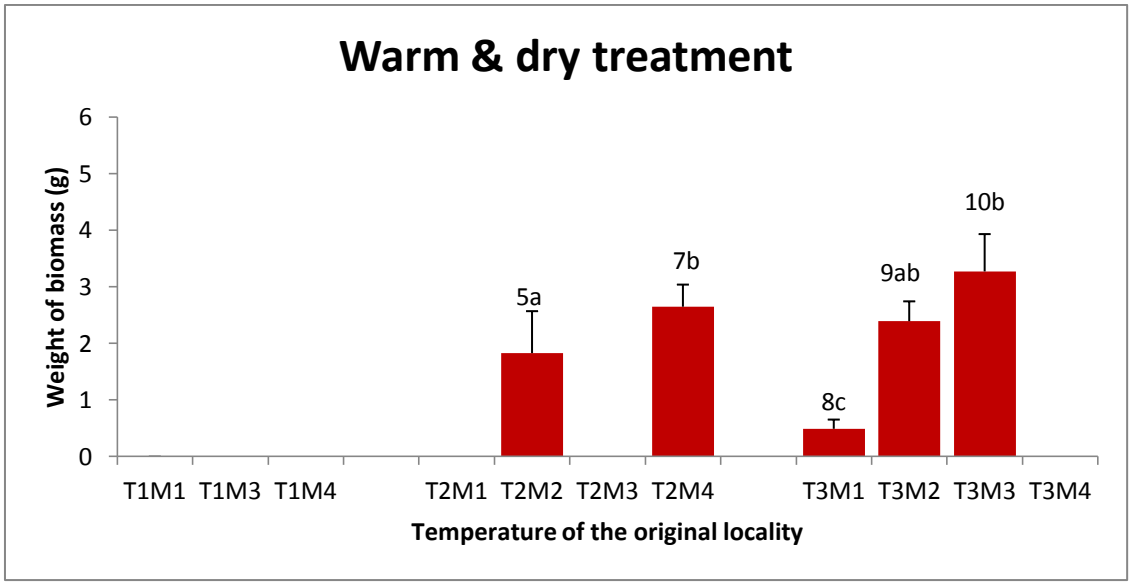

Figure 42 – Weight of biomass in the warm-dry treatment. Columns represent 11 original populations. Height of the column is the mean proportion + SE of biomass of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population. Populations T1M3, T1M4, T2M1 and T2M3 did not have seeds in warm-dry and cold-dry treatments.

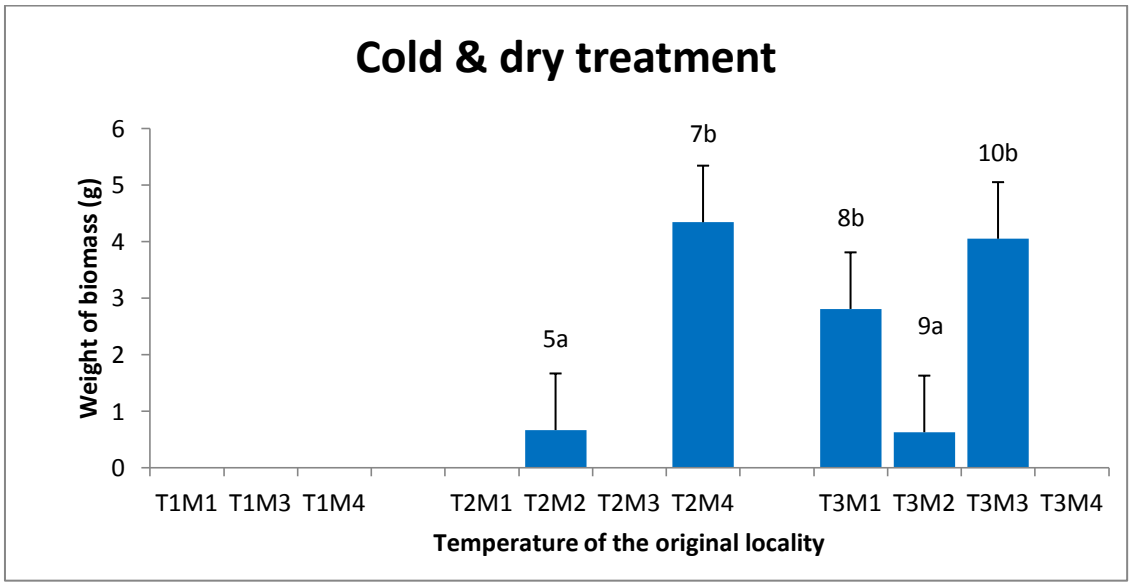

Figure 43 – Weight of biomass in the cold-dry treatment. Columns represent 11 original populations. Height of the column is the mean proportion + SE of biomass of the whole population. On the x axis you can see the combination of the temperature and moisture. T means temperature, T1 is the coldest, T3 the warmest. M means moisture, M1is the driest, M4 the wettest. Columns sharing the same letter are not significantly different from each other. Numbers above the columns represent population. Populations T1M3, T1M4, T2M1 and T2M3 did not have seeds in warm-dry and cold-dry treatments.

3.3 Genetic results

Genetic results were analysed from T1M1 population between 10 pots from each treatment (warm-wet, cold-wet treatment). The analyses done using CCA showed that the effect of mixture of genotypes, treatment and their interaction had significant effect on genotype differences (Tab. 11). Genetic results on the level of single ramets showed significant effect of genotype mixture, effect of treatment and their interaction as well (Tab. 12).

Polysat analyses showed significant differences between treatments (p = 0.001) when ramets were randomised between pots within the genotype mixture. More strict approach with randomising only pots showed p = 0.1537. This result is not significant, but it is quite close to being significant.

Df error = 17	p	explained variability
effect of genotype mixture	**0.040**	8.84%
effect of treatment	**0.036**	9.14%
interaction mixture*treatment	**0.051**	9.28%

Table 11 - Genetic analyses results – on the level of pots, significant results are bold

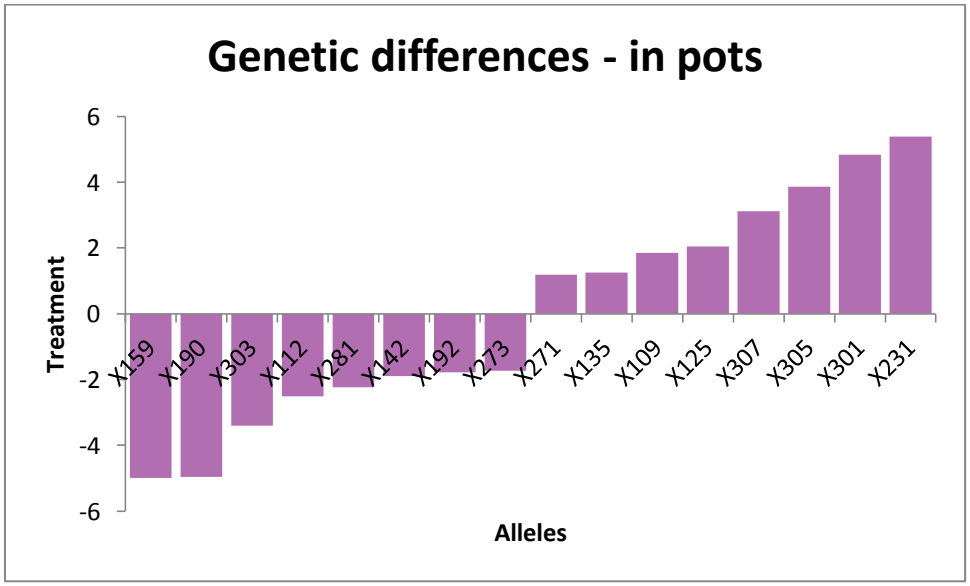

Figure 44 – Graph showing frequency of alleles in 2 treatments determined using DCA analysis – on the level of pots. Above 0 on y axis you can see most frequent alleles in the warm-wet treatment. Below 0 on the y axis you can see most frequent alleles in the cold-wet treatment. X number means code of the allele.

Df error = 341	p	explained variability
effect of genotype mixture	**0.001**	1.82%
effect of treatment	**0.001**	1.88%
interaction mixture*treatment	**0.001**	2.73%

Table 12 - Genetic analyses results – on level of single ramets, significant results are bold

4 Discussion

4.1 Germination characteristics

Results of my study show significant effects of temperature of the growth chamber on the rate of germination of seeds. Seeds from every population germinated better and faster in warm-wet treatment. There were no dormant seeds in this treatment - seeds germinated or got moldy. The second highest proportion of germination was in the cold-wet treatment. Temperature increase can prolong vegetative season if the levels of precipitation are sufficient. In study by Wen *et al.* (2015) when the temperature crossed 35 °C, only quarter of the seeds germinated but failed to form seedlings. Whole-season heating reduced germination and seedlings establishment, significantly in 4 out of 10 species (Shevtsova *et al.* 2009). On the other hand, global warming means increase of temperature to more optimal range for germination for species from higher elevations and latitude. In my study, mean temperature of cold treatments was t = 10.9 °C and in the warm treatments mean temperature was t = 21.3°C. The fastest germination and the greatest proportion of germination was observed in warm-wet treatment in my study, where seeds germinated mostly in the first week. Generally, seeds from colder original areas (T1 and T2) germinated faster and better in the warm-wet treatment. In the study of (Milbau *et al.* 2009), most of the subarctic species also germinated better and faster in warmer treatments (20-22.5°C) than in the cold ones (5-10°C). However, I need to repeat that warm treatments in my study were more like ambient than extremely hot conditions, but considerably warmer than original conditions in Norway for populations from T1 and T2. For populations from T3, the temperature was equal to temperatures during the vegetation season at the localities of their origin.

Results of my study show significant effects of moisture of the climate on the rate of germination of the seeds. Seeds from every population germinated better and faster in wet treatments compared to dry treatments. This means that seeds germinated as long as they had sufficient moisture. Seeds in dry treatments germinated in lower proportions. In the warm-dry treatment, population T1M1 germinated in low proportion but didn't manage to establish

seedlings and population T3M4 didn't germinate at all. Study by Rühl *et al.* (2015) observed germination traits of arable weed species in different temperatures and moisture levels. Their results show significant decrease in mean germination temperature with decreasing moisture. Also study by Wen *et al.* (2015) found that with decreasing moisture germination percentage decreased as well. In global warming scenarios with temperature increase there is a danger of decrease of precipitation in some areas of the world (IPCC 2014).

My results also show significant interaction between temperature and moisture of the growth chamber on germination. This interaction was also in studies Münzbergová *et al.* (2017) and Meineri *et al.* (2013) but not on seed germination but plant growth characteristics and seedling emergence. Seeds that germinated still might die after planting in the pot. For example, population T1M1, in the warm-dry treatment, germinated but didn't established seedlings.

Moisture and temperature of the growth chamber had significant effect on initiation of germination (GI). Initiation of germination was the fastest in warm-wet treatment, thanks to optimal temperature and sufficient moisture. Moisture of the growth chamber had significant effect on germination rate (T_{50}). Seeds germinated significantly faster in warm-wet treatment compared to other regimes in my study, mostly in the first week. Fast germination in warmer conditions is also confirmed by Mondoni *et al.* (2012), where faster germination of seeds was caused by warmer summer and warmer winter season. But they also think that temperature increase only during spring and summer will not have effect on speed of germination. Temperature increase all year round is needed.

Speed of germination is not properly detailed in my study, because I checked the seeds once a week. In the warm-wet and cold-wet treatments, majority of the seeds germinated in the first week. So fastest germination was in those 2 treatments. On some dishes at warm-wet and cold-wet treatments all seeds successfully germinated and they had root longer than 2mm and a green sprout even up to 2 cm long after one week. This suggests that germination started very shortly after first watering. It would be probably better and more time demanding to check seeds in shorter intervals e.g. daily or once in 2 days to capture the speed of germination in highest detail.

In the dry treatment in my study, seeds went dormant but were still able to germinate in optimal conditions months later. Dormancy in cold-dry and warm-dry treatment was higher than in wet treatments. Moisture and temperature of the original population had significant effect on seed dormancy. Moisture of the growth chamber had significant effect on seeds dormancy. In the warm-wet treatment there were no dormant seeds. They either germinated or

get moldy. I noticed that mold was present on the dishes more often and in the greater presence in the warm-wet treatment. Also mould was more aggressive on seeds in warm-wet treatment than in other treatments. It suggests that with higher temperature pathogens spread more quickly and seeds had less chance to germinate or establish a healthy seedling. Like in the study by Graae *et al.* (2009) where higher winter temperatures supported spread of mould. In the study by Edwards *et al.* (2016) dormancy of seeds was also induced by low water potential. Germination and disruption of dormancy are distinctly different processes, and such as, climate change will affect them independently (Walck *et al.* 2011). High proportion of dormant seeds could suggest that plants prefer reproducing through clones over generative reproduction in certain conditions.

4.2 Effects of population origin

Moisture of the original population did not have significant effect on germination rate in my study, but temperature of the original population had significant effect. In my warm-wet treatment, seeds originally from warm localities germinated better. In the study by Blödner *et al.* (2007) seeds originally from the warm environment completed germination faster than those from the cold environment. Another example are seeds of *Piper aduncum*, which showed strong adaptation to local conditions at tropics areas like rapid germination under wet conditions and high temperature tolerance (Wen *et al.* 2015). Results from Cavieres & Arroyo (2000) showed the opposite. I assume that at higher elevations there is lower temperature. In their study seeds from higher elevations, germinated better in 10°C - 20°C than 5°C - 10°C compared to seeds from lower altitudes. But in general seeds originating from warmer-wetter climate populations are often described to germinate at higher rates than populations from colder climate under same conditions (Blödner *et al.* 2007; Schmuths *et al.* 2006). I think that in my results the influence of the original climate of plants is important but not as strong as influence of the actual climate, because *Festuca rubra* can grow in a wide range of conditions and can be very plastic.

To briefly mention, germination can also be influenced by environment experienced by mother plant while producing seeds (Donohue 2005; Chiang *et al.* 2012). During summer (2016) when my plant material was in the experimental garden to reproduce, it was very hot (up to 30°C) and dry. But plants were watered regularly in the garden, so seeds were produced more or less under warm and wet conditions. So it might be natural for seeds to germinate best in warm-wet treatment as well since they were produce under such conditions. All the

populations were grown under same conditions, so there are no differences of effects on mother plants in the garden. Study by Münzbergová & Hadincová (2017) using the same climate grid also demonstrated that maternal conditions may be more important for plant performance than current and original conditions.

Temperature of the original population had significant effect on initiation of germination (GI). Populations from colder original areas (T1, T2) initiated germination the fastest. This suggests that seeds from colder original areas might be adapted to start germinating immediately when the conditions are optimal. They need to establish viable seedlings as soon as possible, because their vegetative season is shorter than in warmer areas.

4.3 Ramets characteristics

Higher density of ramets in my study was in populations from originally more moist sites. Moisture of the original population and moisture of the growth chamber had significant effect on density of ramets. In my study temperature of the growth chamber did not have significant effect on number of ramets. However in other studies temperature of the actual climate had greater significant effect on number of ramets. Temperature was the most important influence and to a lesser extent precipitation on *Arundo donax* ramet recruitment across all months (Decruyenaere & Holt 2005). Their research showed strongly seasonal recruitment and sprouting of ramets. In Zheng *et al.* (2016) number of ramets were significantly correlated with land surface temperature and air temperature. With increasing temperature and moisture, density of ramets increased. And during September when the temperatures dropped, number of viable ramets decreased (Zheng *et al.* 2016). Both mentioned studies Decruyenaere & Holt (2005) and Zheng *et al.* (2016) counted number of ramets of one plant individual, but in my study, I counted all of ramets in one pot. However, I think that increase of temperature and moisture causing increase of number of ramets can be applied to a whole pot as well as one individual. Density of plants in one pot was influenced by how many seeds germinated from the germination part, how many planted seeds established a healthy seedling and how many ramets one plant produced. In my study in the warm-dry and cold-dry treatments mean number of ramets were higher than in wet treatments. I think it is because most of the pots on the watering plate in those treatments were empty, so pots which had seedlings had more light and space next to themselves and could expand more to the width and be more dense. Pots in the warm-wet treatment were not as dense, but plants were higher. Moisture and temperature of the growth chamber had significant effect on the height of plants. Pots in warm-wet

treatment had higher number of planted seeds so pots were already denser than in dry treatments. Thanks to enough of moisture there was high proportion of successful seedlings establishment. This lead to no unoccupied space between pots later, so instead of expanding wider, the plants were forced to grow upwards and be higher to have more light than their neighbor. Also, I noticed that after planting seeds into pots when number of seedlings survived and grew up, it was easier for later planted seeds to also survive. Older seedlings held a small amount of moisture in the soil, so it was easier for younger seedlings to survive. This could be an effect of facilitation. But after approximately 10 weeks from the start of the project plants were big enough to shade each other in the pot or shade neighbouring pots. Making it harder for younger seedlings to grow and harder for smaller and weaker plants to grow big. This could be an effect of competition. Also thanks to germinating earlier in the warm-wet and cold-wet treatments than seeds from dry treatments, seedlings had more time to grow in the pot.

4.4 Selection of genotypes

Genetic analyses of T1M1 in two different treatments showed significant effect of genotype mixture and significant effect of treatment and their interaction. In my study two different treatments supported establishment of different genotypes in identical seed mixtures. This was also demonstrated in the study by Jump *et al.* (2006) and Franks *et al.* (2014). I think that different sensitivity of genotypes to climate change suggests potential of selection of genotypes. To support this idea, Nevo *et al.* (2012) showed allele reduction in different populations of *Triticum dicoccoides* and *Hordeum spontaneum* in different temperatures and moisture levels. Study by Ravenscroft *et al.* (2015) showed that subpopulations of *Festuca ovina* and *Plantago lanceolata* exposed to 15 years of simulated climate change have become genetically differentiated. Jump *et al.* (2008) demonstrated signature of natural selection by drought in the Mediterranean shrub *Fumana thymifolia*. Also study by Wang *et al.* (2017) indicated that heat stress was more detrimental than drought stress for fine fescue species due to greater sensitivity to heat stress and greater genotypic variation of heat tolerance. I cannot deduce effects of drought from my results because I was able to compare only warm-wet and cold-wet treatments of T1M1 population. In the cold-dry chamber seeds from this population did not germinate at all. There were thus no samples to be analyzed. My results showed genetic differences of the same mixture of genotypes and these differences were selected mainly by temperature of the growth chamber.

Approach using allele frequency to analyse genetic differences similar to my study was used in Jump *et al.* (2006) and then pairwise comparisons were used to determine genetic differences between populations. Also, Nevo *et al.* (2012) and Ravenscroft *et al.* (2015) used allele frequency. Alternative approach and the strictest to my result was calculated in Polysat package in R programme using F_{ST} value and it showed not significant differences between treatments when randomising pots with genotypes only from one genotype mixture. Calculating genetic differences comparing to F_{ST} value was used in the study Ravenscroft *et al.* (2015) via 5000 permutations of individuals among climatic subpopulations. Their results were significant.

There are few more ideas of approaching my genetic results that could be considered in the future. First is to recognize single individuals with the same genotype. This way it will reduce more successful seedlings that created more ramets. So the frequency of the alleles will also decrease and might show less significant differences between the two treatments. Second is to look at maternal genotypes, because they are known from previous study, and compare them to genotypes of my generation of plants. This approach would also need to consider paternal genotypes which are from the same population, because pollination was only between individuals from the same population. And third more generations of my plants could be cultivated. But that would be more time demanding and also it would be more complicated. Plants would probably need more space, it would be needed to create a suitable environment for plants to flower and produce seeds and all of this would need to be separated to keep individual populations.

5 Conclusion

The aims of this study were to reveal effect of temperature and moisture on species germination, whether the effect of temperature and moisture depends on the origin of the population and if we can identify signatures of selection from genetically identical seed mixtures under different climate.

Seeds of *Festuca rubra* in my study germinated in greater proportion and faster in warm-wet and cold-wet treatments. Seeds originally from colder areas (T1, T2) germinated better in warm-wet treatment. This suggests probably better germination performance of species originally from colder areas in global warming scenarios. Most of the seeds germinated in warm-wet and cold-wet treatment. Seeds in dry treatments germinated significantly slower and less or went into dormancy. Even if the seed germinated, successful establishment of seedling wasn't guaranteed. Wet treatments had higher plants but lower density of ramets and dry treatments had shorter plants but higher number of ramets in one pot.

Temperature of the original population had significant effect on rate of seeds germination. Moisture of the original population did not have significant effect on germination rate in my study. Seeds originally from warm localities germinated better. The influence of the original population is important but not as strong as the actual climate.

There was significant difference in genotypes of one seed mixture between different climates. This suggests potential selection of genotypes under different climate, but further research is required.

6 Literature

Baskin, C.C. & Baskin, J.M., 2014. *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination,* Second edition., Academic Press.

Beniston, M., 2003. Climatic Change in Mountain Regions : *Climate Change*, 59, pp.5–31.

Beniston, M., Diaz, H.F. & Bradley, R.S., 1997. Climatic change at high elevation sites: An overview. *Climatic Change*, 36, pp.233–251.

Bernareggi, G. *et al.*, 2015. Climate warming could increase seed longevity of alpine snowbed plants. *Alpine Botany*, 125(2), pp.69–78.

Besnard, G. *et al.*, 2008. Polyploidy in the Olive Complex (Olea europaea): Evidence from Flow Cytometry and Nuclear Microsatellite Analyses. *Annals of Botany*, 101(1), pp.25–30.

Bewley, J., 1997. Seed Germination and Dormancy. *The Plant cell*, 9(7), pp.1055–1066.

Blödner, C. *et al.*, 2007. Warm and cold parental reproductive environments affect seed properties, fitness, and cold responsiveness in Arabidopsis thaliana progenies. *Plant, Cell and Environment*, 30(2), pp.165–175.

Bresson, C.C. *et al.*, 2011. To what extent is altitudinal variation of functional traits driven by genetic adaptation in European oak and beech? *Tree Physiology*, 31(11), pp.1164–1174.

Cavieres, L.A. & Arroyo, M.T.K., 2000. Seed Germination Response to Cold Stratification Period and Thermal Regime in Phacelia secunda (Hydrophyllaceae): Altitudinal Variation in the Mediterranean Andes of Central Chile. *Plant Ecology*, 149(1), pp.1–8.

Cottrell, H.J., 1947. Tetrazolium salt as a seed germination indicator. *Nature*, 159(4048), p.748.

Decruyenaere, J.G. & Holt, J.S., 2005. Ramet demography of a clonal invader, Arundo donax (Poaceae), in Southern California. *Plant and Soil*, 277(1-2), pp.41–52.

Dirihan, S. *et al.*, 2016. Geographic Variation in Festuca rubra L . Ploidy Levels and Systemic Fungal Endophyte Frequencies. *PLoS ONE*, 11(11).

Donohue, K., 2005. Seeds and seasons: interpreting germination timing in the field. *Seed*

science research, 15(3), pp.175–187.

Edwards, B.R. *et al.*, 2016. Maternal temperature effects on dormancy influence germination responses to water availability in Arabidopsis thaliana. *Environmental and Experimental Botany*, 126, pp.55–67.

Fay, P.A. & Schultz, M.J., 2009. Germination, survival, and growth of grass and forb seedlings: Effects of soil moisture variability. *Acta Oecologica*, 35(5), pp.679–684.

Fenner, M. & Thompson, K., 2005. *The Ecology of Seeds*, New York: Cambridge University Press.

Fernandez-Pascual, E., Seal, C.E. & Pritchard, H.W., 2015. Simulating the germination response to diurnally alternating temperatures under climate change scenarios: comparative studies on Carex diandra seeds. *Annals of Botany*, 115(2), pp.201–209.

Franks, S.J., Weber, J.J. & Aitken, S.N., 2014. Evolutionary and plastic responses to climate change in terrestrial plant populations. *Evolutionary Applications*, 7(1), pp.123–139.

Fu, Y.B. *et al.*, 2006. Characterization of microsatellite markers for rough fescue species (Festuca spp.). *Molecular Ecology Notes*, 6(3), pp.894–896.

Gao, S.B. *et al.*, 2018. Phenotypic plasticity vs. local adaptation in quantitative traits differences of Stipa grandis in semi-arid steppe, China. *Scientific Reports*, 8(1), pp.1–8.

Ghalambor, C.K. *et al.*, 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, (21), pp.394–407.

Gienapp, P. *et al.*, 2008. Climate change and evolution : disentangling environmental and genetic responses. *Molecular Ecology*, (17), pp.167–178.

Gordon, D.R. & Rice, K.J., 1998. Patterns of differentiation in wiregrass (Aristida beyrichiana): implications for restoration efforts. *Restoration Ecology*, 6(2), pp.166–174.

Graae, B.J. *et al.*, 2009. The effect of an early-season short-term heat pulse on plant recruitment in the Arctic. *Polar Biology*, 32(8), pp.1117–1126.

Graae, B.J., Alsos, I.G. & Ejrnaes, R., 2008. The impact of temperature regimes on development, dormancy breaking and germination of dwarf shrub seeds from arctic,

alpine and boreal sites. *Plant Ecology*, 198(2), pp.275–284.

Harsch, M.A. *et al.*, 2009. Are treelines advancing? A global meta-analysis of treeline response to climate warming. *Ecology Letters*, 12(10), pp.1040–1049.

Heydecker, W. & Orphanos, P.I., 1968. The effect of excess moisture on the germination of Spinacia oleracea L. *Planta*, 83(3), pp.237–247.

Houghton, J., 1998. *Globální oteplování: Úvod do studia změn klimatu a prosředí*, Praha: Academia.

Chen, I., 2012. Rapid Range Shifts of Species. *Science*, 1024(2011), pp.17–20.

Chiang, G.C. *et al.*, 2012. Pleiotropy in the wild: The dormancy gene DOG1 exerts cascading control on life cycles. *Evolution (N. Y).*, 67, pp.883–893.

IPCC, 2014. *Climate Change 2013 - The Physical Science Basis* Intergovernmental Panel on Climate Change, ed., Cambridge: Cambridge University Press.

Jump, A.S. *et al.*, 2006. Natural selection and climate change: Temperature-linked spatial and temporal trends in gene frequency in Fagus sylvatica. *Molecular Ecology*, 15(11), pp.3469–3480.

Jump, A.S. *et al.*, 2008. Simulated climate change provokes rapid genetic change in the Mediterranean shrub Fumana thymifolia. *Global Change Biology*, 14(3), pp.637–643.

Jump, A.S. & Peñuelas, J., 2005. Running to stand still: Adaptation and the response of plants to rapid climate change. *Ecology Letters*, 8(9), pp.1010–1020.

Kelly, C. *et al.*, 2003. Temperature-based population segregation in birch. *Ecology Letters*, pp.87–89.

Körner, C., 2003. *Alpine Plant Life*, Berlin, Heidelberg: Springer Berlin Heidelberg.

Koti, S. *et al.*, 2005. Interactive effects of carbon dioxide , temperature , and ultraviolet-B radiation on soybean (Glycine max L.) flower and pollen morphology, pollen production, germination, and tube lengths. , 56(412), pp.725–736.

Lauvergeat, V. *et al.*, 2005. Sixty simple sequence repeat markers for use in the Festuca–Lolium complex of grasses. *Molecular Ecology Notes*, 5(2), pp.401–405.

Lenoir, J. *et al.*, 2008. A Significant Upward Shift in Plant Species Optimum Elevation During the 20th Century. *Science*, 320(5884), pp.1768–1771.

Levin, D. a., 2003. Ecological speciation: lessons from invasive species. *Systematic Botany*, 28(4), pp.643–650.

Liu, K. *et al.*, 2011. Effect of storage conditions on germination of seeds of 489 species from high elevation grasslands of the eastern Tibet Plateau and some implications for climate change. *American Journal of Botany*, 98(1), pp.12–19.

Liu, Y. *et al.*, 2014. Synergistic and antagonistic effects of salinity and pH on germination in switchgrass (Panicum virgatum L.). *PLoS ONE*, 9(1), pp.23–25.

Lloret, F., Penuelas, J. & Estiarte, M., 2004. Experimental evidence of reduced diversity of seedlings due to climate modification in a Mediterranean-type community. *Global Change Biology*, 10(2), pp.248–258.

Martin, R.E., Asner, G.P. & Sack, L., 2007. Genetic variation in leaf pigment, optical and photosynthetic function among diverse phenotypes of Metrosideros polymorpha grown in a common garden. *Oecologia*, 151(3), pp.387–400.

McCabe, G.J. & Wolock, D.M., 2010. Long-term variability in Northern Hemisphere snow cover and associations with warmer winters. *Climatic Change*, 99(1), pp.141–153.

McNeely, J.A. *et al.*, 1990. *Conserving the world's bilogical diversity*, International Union for Conservation of Nature and Natural Resources.

Meineri, E., Spindelböck, J. & Vandvik, V., 2013. Seedling emergence responds to both seed source and recruitment site climates: A climate change experiment combining transplant and gradient approaches. *Plant Ecology*, 214(4), pp.607–619.

Michel, B.E., 1983. Evaluation of the water potentials of solutions of polyethylene glycol 8000 both in the absence and presence of other solutes. *Plant physiology*, 72(1), pp.66–70.

Milbau, A. *et al.*, 2009. Effects of a warmer climate on seed germination in the subarctic. *Annals of Botany*, 104(2), pp.287–296.

Mondoni, A. *et al.*, 2015. Climate warming could increase recruitment success in glacier

foreland plants. *Annals of Botany*, 116(6), pp.907–916.

Mondoni, A. *et al.*, 2012. Climate warming could shift the timing of seed germination in alpine plants. *Annals of Botany*, 110(1), pp.155–164.

Morin, X. & Thuiller, W., 2009. Comparing Niche and Process Based Models to Reduce Prediction Uncertainty in Species Range Shifts under Climate Change, *Ecology*, 90(5), pp.1301–1313.

Müller, E., Cooper, E.J. & Alsos, I.G., 2011. Germinability of arctic plants is high in perceived optimal conditions but low in the field. *Botany*, 89(5), pp.337–348.

Münzbergová, Z. *et al.*, 2017. Genetic differentiation and plasticity interact along temperature and precipitation gradients to determine plant performance under climate change *Journal of Ecology*, 105(5), pp.1358–1373.

Münzbergová, Z. & Hadincová, V., 2017. Transgenerational plasticity as an important mechanism affecting response of clonal species to changing climate. *Ecology and Evolution*, 7(14), pp.5236–5247.

Nevo, E. *et al.*, 2012. Evolution of wild cereals during 28 years of global warming in Israel. *Israel journal of plant sciences*, 109(13), pp.3412–3415.

Nicotra, A.B. *et al.*, 2010. Plant phenotypic plasticity in a changing climate. *Trends in Plant Science*, 15(12), pp.684–692.

NSIDC, 2018. "All About Arctic Climatology and Meteorology." National Snow and Ice Data Center. Available at: nsidc.org/cryosphere/arctic-meteorology/index.html. [Accessed July 9, 2018].

Parmesan, C., 2016. Ecological and Evolutionary Responses to Recent Climate Change. *Annual Review of Ecology Evolution and Systematics*, 37(2006), pp.637–669.

Parolo, G. & Rossi, G., 2008. Upward migration of vascular plants following a climate warming trend in the Alps. *Basic and Applied Ecology*, 9(2), pp.100–107.

Pérez-Fernández, M.A., Calvo-Magro, E. & Ferrer-Castán, D., 2006. Simulation of germination of pioneer species along an experimental drought gradient. *Journal of Environmental Biology*, 27(4), pp.679–685.

Pfeiffer, T. *et al.*, 2011. Characterization of Microsatellite Loci and Reliable Genotyping in a Polyploid Plant, Mercurialis perennis (Euphorbiaceae). *Journal of Heredity*, 102(4), pp.479–488.

Qaderi, M.M. & Cavers, P.B., 2002. Interpopulation and interyear variation in germination in Scotch thistle, Onopordum acanthium L., grown in a common garden: Genetics vs environment. *Plant Ecology*, 162(1), pp.1–8.

Ravenscroft, C.H., Whitlock, R. & Fridley, J.D., 2015. Rapid genetic divergence in response to 15 years of simulated climate change. *Global Change Biology*, 21(11), pp.4165–4176.

Riordan, E.C. *et al.*, 2016. Association of genetic and phenotypic variability with geography and climate in three southern California oaks. *American Journal of Botany*, 103(1), pp.73–85.

Rühl, A.T. *et al.*, 2015. Future challenge for endangered arable weed species facing global warming: Low temperature optima and narrow moisture requirements. *Biological Conservation*, 182, pp.262–269.

Sadeghi, H. *et al.*, 2011. Effect of seed osmopriming on seed germination behavior and vigor of soybean (Glycine max L). *Network*, 6(1), pp.39–43.

Sampoux, J. & Huyghe, C., 2009. Contribution of ploidy-level variation and adaptive trait diversity to the environmental distribution of taxa in the “fine-leaved fescue” lineage (genus Festuca subg . Festuca). *Journal of Biogeography*, pp.1978–1993.

Shevtsova, A. *et al.*, 2009. Critical periods for impact of climate warming on early seedling establishment in subarctic tundra. *Global Change Biology*, 15(11), pp.2662–2680.

Schmuths, H. *et al.*, 2006. Effects of preconditioning and temperature during germination of 73 natural accessions of Arabidopsis thaliana. *Annals of Botany*, 97(4), pp.623–634.

Springfield, H.W., 1968. Germination of Winterfat Seeds under Different Moisture Stresses and Temperatures. *Journal of Range Management*, 21(5), p.314.

Still, D.W., Kim, D.H. & Aoyama, N., 2005. Genetic variation in Echinacea angustifolia along a climatic gradient. *Annals of Botany*, 96(3), pp.467–477.

Sultan, S.E. & Spencer, H.G., 2002. Metapopulation Structure Favors Plasticity over Local

Adaptation. *The American Naturalist*, 160(2), pp.271–283.

Šurinová, M., Hadincová, V. & Münzbergová, Z., Genetic differentiation of Festuca rubra populations climatic grid., Submitted

Teixeira, H., Rodríguez-Echeverría, S. & Nabais, C., 2014. Genetic Diversity and Differentiation of Juniperus thurifera in Spain and Morocco as determined by SSR. *PLoS ONE*, 9(2).

De Vitis, M. *et al.*, 2014. Rapid adaptation of seed germination requirements of the threatened Mediterranean species Malcolmia littorea (Brassicaceae) and implications for its reintroduction. *South African Journal of Botany*, 94, pp.46–50.

Walck, J.L. *et al.*, 2011. Climate change and plant regeneration from seed. *Global Change Biology*, 17(6), pp.2145–2161.

Walder, T. & Erschbamer, B., 2015. Temperature and drought drive differences in germination responses between congeneric species along altitudinal gradients. *Plant Ecology*, 216(9), pp.1297–1309.

Walther, G.-R. *et al.*, 2002. Ecological responses to recent climate change. *Nature*, 416, pp.389–395.

Wang, J. *et al.*, 2017. Differential Physiological Responses and Genetic Variations in Fine Fescue Species for Heat and Drought Stress. *Journal of the American Society for Horticultural Science*, 142(5), pp.367–375.

Wen, B. *et al.*, 2015. Seed germination of the invasive species Piper aduncum as influenced by high temperature and water stress. *Weed Research*, 55(2), pp.155–162.

Wetherald, R.T., 2002. Simulation of hydrologic changes associated with global warming. *Journal of Geophysical Research*, 107(D19), p.4379.

Zheng, Y. *et al.*, 2016. Clonal growth of Populus Pruinosa Schrenk and its role in the regeneration of riparian forests. *Ecological Engineering*, 94, pp.380–392.